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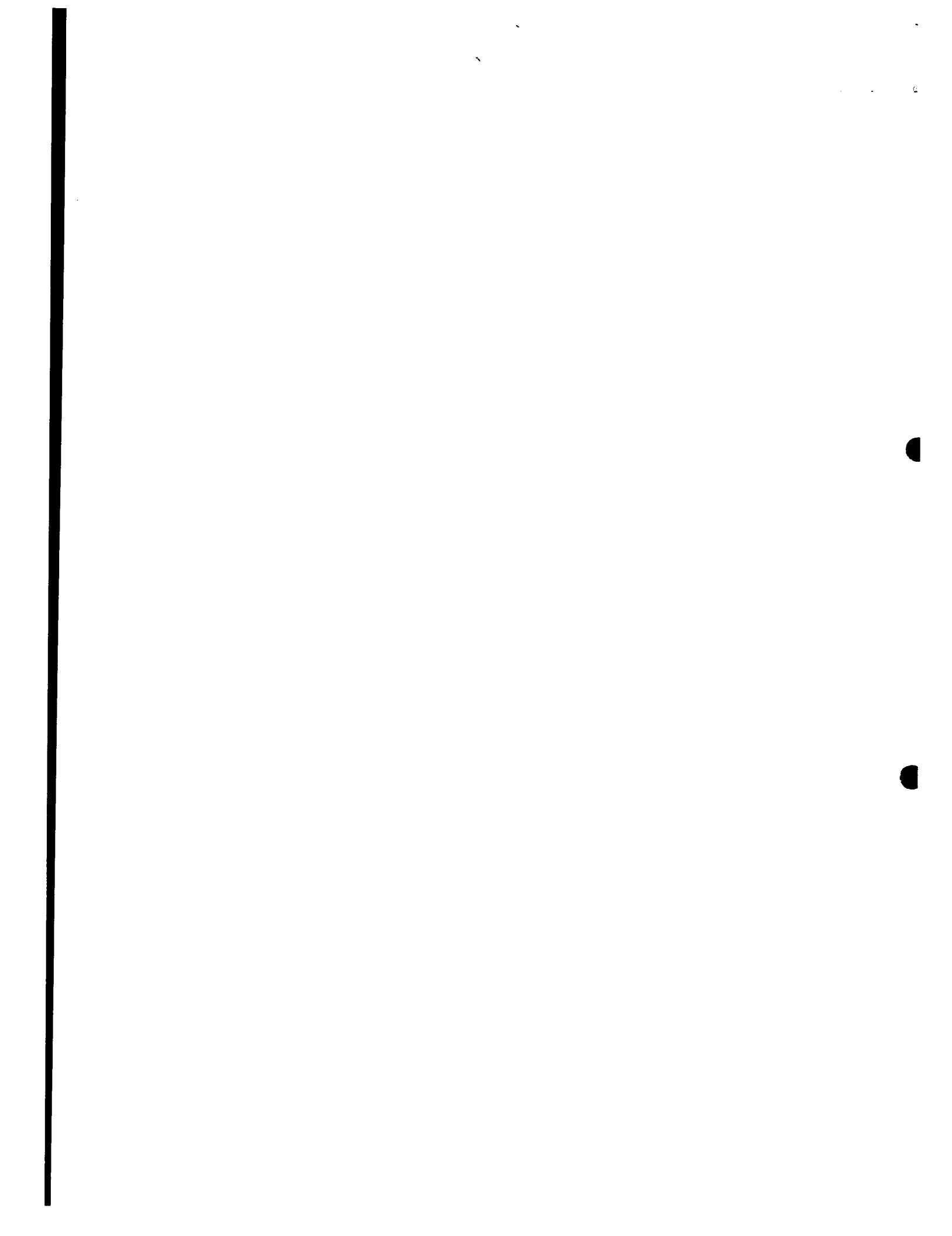
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Recombinant soluble Fc receptors

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Recombinant soluble Fc receptors

Specification

5

- The present invention relates to recombinant soluble Fc receptors (FcR), recombinant nucleic acids coding for such Fc receptors, host cells containing corresponding nucleic acids as well as a process for the determination of the amount of antibodies of a certain type contained in the blood, plasma or serum of a patient, a process for the determination of the immune status of patients with chronic diseases of the immune system and a process for the screening of substances in view of their ability to act as inhibitors of the recognition and binding of antibodies to the respective cellular receptors. Further, the present invention is concerned with pharmaceutical compositions containing the recombinant soluble FcRs, the use of a crystalline preparation of recombinant soluble FcRs for the generation of crystal structure data of Fc receptors as well as FcR inhibitors and pharmaceutical compositions containing such FcR inhibitors.
- 10 A still further subject of the present invention is a recombinant Fc receptor coupled to a solid phase, e.g. a chromatography carrier material. The use of such chromatography material, which is another subject of the present invention, lies in the absorption of immunoglobulins from a body fluid of patients or from culture supernatants of immunoglobulin producing cells.
- 15
- 20
- 25 Fc receptors (FcRs) play a key role in defending the human organism against infections. After pathogens have gained access to the blood circulation they are opsonized by immunoglobulins (Igs). The resulting immunocomplexes bind due to their multivalency with high avidity to FcR bearing cells leading to clustering of the FcRs, which triggers several effector functions (Metzger, H., 1992A). These include, depending on the expressed FcR type and associated proteins, endocytosis with subsequent neutralization of the
- 30

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pathogens and antigen presentation, antibody-dependent cellular cytotoxicity (ADCC), secretion of mediators or the regulation of antibody production (Fridman et al, 1992; van de Winkel and Capel, 1993).

- 5 Specific FcRs exist for all Ig classes, the ones for IgG being the most abundant with the widest diversity. Together with the high affinity receptor for IgE (Fc ϵ RIa), Fc γ RI (CD64), Fc γ RII (CD32) and Fc γ RIIIa (CD16) occur as type I transmembrane proteins or in soluble forms (sFcRs) but also a glycosylphosphatidylinositol anchored form of the Fc γ RIII (Fc γ RIIIb) exists.
- 10 Furthermore, Fc γ Rs occur in various isoforms (Fc γ RIa, b1, b2, c; Fc γ RIIa1-2, b1-3, c) and alleles (Fc γ RIIa1-HR, -LR; Fc γ RIIIb-NA1,-NA2) (van de Winkel and Capel, 1993). In contrast to the overall homologous extracellular parts, the membrane spanning and the cytoplasmic domains differ. They may be deleted entirely or be of a size of 8 kDa. They may contain either a 26 amino acid immunoreceptor tyrosine-based activation motif (ITAM) as in Fc γ RIIa or a respective 13 amino acid inhibitory motif (ITIM) in Fc γ RIIb involved in signal transduction (Amigorena et al, 1992).
- 15

- Judged by the conserved spacing of cysteins, the extracellular part of the
- 20 FcRs consists of three (Fc γ RI, CD64) or two (Fc ϵ RI, Fc γ RII, CD32 and Fc γ RIII, CD16) Ig-like domains (10 kDa/domain) and therefore belongs to the immunoglobulin super family. These highly glycosylated receptors are homologues, and the overall identity in amino acid sequence among the Fc γ Rs and Fc ϵ RIa exceeds 50% in their extracellular regions. Nevertheless,
- 25 the affinity of FcRs to their ligands varies widely. The higher affinity of $\approx 10^8 M^{-1}$ of the Fc γ RI to Fc-fragment is assigned to its third domain, while the other Fc γ Rs with two domains have an affinity to IgG varying between 10^5 and $10^7 M^{-1}$. The affinity of the two domain Fc ϵ RIa to IgE exceeds these values by far with a constant of $10^{10} M^{-1}$ (Metzger, H., 1992B).
- 30 Fc γ Rs are expressed in a defined pattern on all immunological active cells. Fc γ RI is constitutively expressed on monocytes and macrophages and can be induced on neutrophils and eosinophils. The physiological role of Fc γ RI

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is still unknown as the expression on monocytes is not vital (Ceuppens et al, 1988). The GPI anchored form of Fc γ RIII (Fc γ RIIib) is exclusively expressed on granulocytes. Due to its missing cytoplasmic part, the signal transduction into the cell occurs solely via other transmembrane proteins
5 like complement receptor type 3 (CR3) that can at least associate with Fc γ RIIib (Zhou et al, 1993; Poo et al, 1995). Fc γ RIIa is mainly expressed on monocytes and macrophages but only in conjunction with associated proteins (e.g. α - or γ -chains). Fc γ RII is the receptor with the widest distribution on immunocompetent cells and is mainly involved in the
10 endocytosis of immunocomplexes.

Fc γ RIIa and Fc γ RIIb differ in their extracellular region by only 7% of the amino acid residues. Nevertheless, both forms can be distinguished by their binding characteristics to human and mouse IgG subclasses (van de Winkel and Capel, 1993) and their differing affinity to human IgGs (Sondermann et al, 1998A). The situation is rendered even more complicated by the high responder/low responder (HR/LR) polymorphism of Fc γ RIIa named after the ability of T cells from some individuals to respond to murine IgG1-induced mitogenesis (Tax et al, 1983). Later, it was found that the two exchanges
15 in the amino acid sequence between the LR and the HR form modify the ability to bind human IgG2, which leads to the suggestion that at least one of them is involved in IgG binding (Hogarth et al, 1992).

In contrast to the beneficial role FcRs play in the healthy individual, they
25 also transmit the stimulation of the immune system in allergies (Fc ϵ RIa) or autoimmune diseases. Moreover, some viruses employ Fc γ Rs to get access to cells like HIV (Homsy et al, 1989) and Dengue (Littaua et al, 1990) or slow down the immune response by blocking Fc γ Rs as in the case of Ebola
30 (Yang et al, 1998) and Measles (Ravanel et al, 1997).

Hence, the object underlying the present invention was to provide receptors which are easy to produce and can advantageously be used for medical or

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diagnostic applications. Moreover, it was an object of the invention to provide soluble receptors exhibiting a binding specificity and activity which is analogous to that of the receptors occurring naturally in the human body and which, additionally, make it possible to produce crystals suitable for a structure determination.

This object is accomplished by recombinant soluble Fc receptors which consist only of the extracellular portion of the receptor and are not glycosylated. The receptors according to the present invention are therefore characterized by the absence of transmembrane domains, signal peptides and glycosylation.

Particularly preferred for the present invention are Fc γ or Fc ϵ receptors. This is because IgG and IgE molecules are characteristic for a multiplicity of diseases and conditions, so that their determination and possible ways of influencing them are of great interest. Figure 8 shows an alignment of amino acid sequences of the extracellular parts of some Fc γ Rs and Fc ϵ Rs. The FcRs according to the invention include all these sequences or parts thereof that still retain binding capacity to antibodies and/or proper crystallization.

In a particularly preferred embodiment of the invention the recombinant soluble FcR is a Fc γ RIIb receptor. Further, it is particularly preferred that the receptor be of human origin. In a particularly preferred embodiment, it contains an amino acid sequence as shown in SEQ ID NO:1 or SEQ ID NO:2.

According to the present invention, the preparation of the soluble Fc receptors preferably takes place in prokaryotic cells. After such expression, insoluble inclusion bodies containing the recombinant protein form in prokaryotic cells, thus facilitating purification by separation of the inclusion bodies from other cell components before renaturation of the proteins

- 5 -

- contained therein takes place. The renaturation of the FcRs according to the present invention which are contained in the inclusion bodies can principally take place according to known methods. The advantage of the preparation in prokaryotic cells, the production of inclusion bodies and the thus obtained recombinant soluble Fc receptors make it possible to obtain a very pure and, in particular, also very homogeneous FcR preparation. Also because of the absence of glycosylation the obtained product is of great homogeneity.
- 10 Soluble Fc receptors hitherto produced by recombinant means particularly exhibited the disadvantage that a much more elaborate purification was required, since they were expressed in eukaryotic cells and, due to the glycosylation which is not always uniform in eukaryotic cells, these products were also less homogeneous.
- 15 The recombinant soluble Fc receptors according to the present invention even make it possible to produce crystals suitable for use in X-ray analysis, as shall be explained lateron. The FcRs of the present invention moreover exhibit practically the same activity and specificity as the receptors naturally occurring in vivo.
- 20 A further subject matter of the present invention is a recombinant nucleic acid having a sequence coding for a recombinant soluble Fc receptor according to the present invention.
- 25 The nucleic acid according to the present invention may contain only the coding sequences or, additionally, vector sequences and, in particular, expression control sequences operatively linked to the sequence encoding the recombinant FcR, like promoters, operators and the like.
- 30 In a particularly preferred embodiment the nucleic acid of the present invention contains a sequence as shown in SEQ ID NO:3 or SEQ ID NO:4.

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In these sequence protocols the atg start codons are in bold print and newly introduced restriction sites are underlined. For a comparison, SEQ ID NO:5 and SEQ ID NO:6 show the respective wild type sequences coding for Fc γ RIIb and Fc ϵ Rla. SEQ ID NOs:7-9 show the wild type sequences for
5 Fc γ RI, Fc γ RIIa and Fc γ RIII, which can be modified in a similar way as SEQ ID NO:3 and SEQ ID NO:4 to obtain FcRs according to the invention.

If the nucleic acid of the present invention contains vector sequences, then
these are preferably sequences of one or several prokaryotic expression
10 vectors, preferably of pET vectors. Any other known functions of
expression vectors may also be contained in the recombinant nucleic acid
according to the present invention if desired. These may, for instance, be
resistance genes allowing for an effective selection of transformed host
cells.

15

A still further subject matter of the present invention is a host cell
containing a recombinant nucleic acid according to the present invention.
As repeatedly mentioned above, the host cell preferably is a prokaryotic
host cell, particularly an E. coli cell.

20

The recombinant soluble Fc receptors according to the present invention
can be used for a multitude of examinations or applications because they
specifically react with antibodies. In vivo, the soluble Fc receptors are
powerful immunoregulators which, if present in elevated levels, result in a
25 remarkable suppression of the immune system which leads to many partly
known and partly not yet understood effects. Based on these effects,
several applications of the Fc receptors according to the present invention
are further subject matters of the present invention.

30 One such subject is a process for the determination of the amount of
antibodies of a certain type in the blood or serum of a patient, which is
characterized by the use of a recombinant soluble FcR according to the

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invention in an immunoassay, and the determination of the presence of FcR-antibody complexes. Such assay allows to screen for the presence of a certain kind of antibody and allows also for the determination of the amount of antibodies present in the blood, plasma or serum of a patient.

5

Any type of immunoassay is principally suitable for the use according to the present invention, as long as the presence of FcR-antibody complexes can thereby be detected. Both ELISA (enzyme-linked immunosorbent immunoassay), particularly sandwich assays, and RIA (radio-immunoassay) 10 are suitable, but also competitive testing methods. In a preferred embodiment of the invention where the presence and/or the amount of IgE antibodies is to be examined, an Fc ϵ R is used as recombinant soluble receptor according to the present invention. In particular, this method is suited and advantageous for determining a predisposition or manifestation 15 of an allergy.

Moreover, a method is preferred in which the presence of soluble FcRs is to be determined and, if required, quantified, an Fc γ R being used as recombinant soluble receptor according to the invention. By means of this 20 test among others the immune status of patients with chronic diseases of the immune system can be determined in a competitive immunoassay. Chronic diseases in the sense of these processes are for instance AIDS, SLE (systemic lupus erythematosus), MM (multiple myeloma) or rheumatoid arthritis.

25

A further advantageous use of the receptor according to the present invention lies in the screening of substances in view of their ability to act as inhibitors of the recognition and binding of antibodies to the respective cellular receptors.

30

By means of modern screening techniques such as HTPS (high throughput screening) in combination with multi-well microtiter plates and automatic

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pipetting apparatuses it is nowadays possible to simultaneously test a multitude of substances for specific properties. As the FcRs according to the present invention can be easily produced at low cost, they can also be used in such series tests by which substances having an inhibiting effect
5 can easily be identified.

Particularly preferred is such use according to which Fc receptors according to the present invention are used to find inhibitors capable of inhibiting the recognition and binding of the respective antibodies to the particular
10 receptor of interest.

A further area of application of the substances according to the invention lies in the pharmaceutical field. Hence, a further subject matter of the invention is a pharmaceutical composition comprising as active agent a recombinant soluble FcR according to the invention. According to the
15 present invention, this pharmaceutical composition may of course comprise conventional useful carrier and auxiliary substances. Such substances are known to the person of skill in the art, the mode of administration also having to be taken into account. The pharmaceutical composition of the
20 present invention can be advantageously used for the treatment or prevention of autoimmune diseases, allergies or tumor diseases.

Soluble forms of Fc receptors such as FcγRIII mediate isotype-specific regulation of B cell growth and immunoglobulin production. In a murine
25 model of myeloma, sFcR suppresses growth and immunoglobulin production of tumor cells (Müller et al, 1985; Roman et al, 1988; Teillaud et al, 1990). Furthermore, sFcR binds to surface IgG on cultures of human IgG-secreting myeloma cells and effects suppression of tumor cell growth and IgG secretion. Prolonged exposure of these cells to sFcR results in tumor cell
30 cytolysis (Hoover et al, 1995).

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Also, overreactions of the immune system in allergic reactions or due to massive antigen load might be reduced by, for example, intravenous application of soluble FcR (Ierino et al, 1993).

- 5 Therefore, a preferred pharmaceutical composition according to the invention for use in the treatment of AIDS, rheumatoid arthritis or multiple myeloma contains a recombinant soluble Fcy receptor and, preferably, a receptor having the amino acid sequence as shown in SEQ ID NO:1.
- 10 It was also of great interest to obtain crystal structure data of Fc receptors. On the one hand, these are a key to the understanding of molecular mechanisms in immunocomplex recognition. On the other hand, these structural data can be used to find out common features in the structures of different Fc receptors and use the knowledge of the structures to generate inhibitors or identify and produce new artificial antibody receptors.
- 15

To obtain such crystal structure data, a crystalline preparation of the recombinant soluble Fc receptor according to the invention is used. The recombinant soluble FcRs according to the invention surprisingly can be 20 obtained pure enough to produce crystals that give reliable X-ray structure determination data. Such crystallization was not possible with the hitherto produced receptor molecules, mostly due to their lack of homogeneity.

The stated applications are merely preferred embodiments of the use of the 25 crystal structure data. Many other applications seem possible, too.

Suitably, the structural data for the generation and/or identification of 30 inhibitors or new receptors, respectively, are used in a computer-aided modelling program. Software for computer-aided modelling is available to the man skilled in the art. That application is already described for structure identification or design of other substances.

- 10 -

Particularly preferred for the present invention are the structures as shown in the enclosed Examples and Figures for the respective receptors. Such structures can be used to design inhibitors, antagonists and artificial receptor molecules.

5

A still further subject matter of the present invention, therefore, is a FcR inhibitor which has a three-dimensional structure which is complementary to the recombinant soluble FcR according to the invention and inhibits the binding of antibodies to FcRs.

10

What is important for the inhibitors of the invention is that, owing to their structure and specificity, they are capable of binding to the FcRs and thus prevent their normal binding to the constant parts of antibodies.

15

Preferably, such FcR inhibitors are small organic molecules which can easily be administered orally. They might be an interesting alternative to cortisone in the treatment of autoimmune diseases and host/graf rejections. Such a molecule would also suppress reinfection rates with certain viruses, e.g. Dengue virus where the antibody coated virus is Fc γ RIIb dependent internalized (Littaua et al, 1990), HIV where on CD4 positive T cells an antibody enhancement of HIV infection is mediated by Fc γ RIII (Homsy et al, 1989), or Ebola where the virus secreted glycoprotein inhibits early neutrophil activation by blocking sFc γ RIII which affects the host response to infection (Yang et al, 1998).

20

The development of inhibitors might also lead to substances that interfere with the recognition of IgE by their receptors. From the modelled structure of Fc ϵ RI, peptides have already been developed which inhibit mast cell degranulation in vitro. With the knowledge of the structures of the receptor or the receptor-antibody complex in atomic detail, a new possibility for a rational drug design is opened.

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A further subject matter of the present invention therefore is a pharmaceutical composition containing as active agent an FcR inhibitor as mentioned above. Such pharmaceutical compositions may, for example, be used in the treatment or prevention of diseases which are due to overreactions or faulty reactions of the immune system, preferably the treatment or prevention of allergies, autoimmune diseases or anaphylactic shock.

A further subject of the present invention is the sFcR according to the invention, bound to a solid phase. Such heterogeneous receptors might be used for immunoassays or other applications where the receptor in an immobilized form can be used beneficially.

In a preferred embodiment of the invention the solid phase is a chromatography carrier material onto which the Fc receptor is fixed, e.g. sepharose, dextran sulfate etc. Such chromatography materials with Fc receptors bound thereto can beneficially be used for the adsorption of immunoglobulins from the blood, plasma or serum of patients or from the culture supernatant of immunoglobulin producing cells (meaning concentration, enrichment and purification of antibodies).

On the one hand, the antibodies bound to the chromatography material can be eluted and, for example, the immune status of a patient can thereby be determined. On the other hand, antibodies from the blood of a patient can thereby be enriched before carrying out further tests, which is a further preferred embodiment of the present invention. In many cases it is difficult to conduct diagnostic assays using blood samples if the latter contains only a very small number of the antibodies to be identified. By means of a concentration using a specific chromatographic column with Fc receptors according to the present invention, antibodies of interest can easily be concentrated and separated from many other substances which might disturb the test.

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Basically, it is also possible to use a chromatography material according to the present invention in an extracorporeal perfusion system for lavage of the blood in case of certain diseases where the removal of antibodies plays a crucial role.

5

The following Examples are to further illustrate the invention in conjunction with the Figures.

Example 1

10

1.1 Cloning and Expression

The cDNA of human Fc γ RIIb2 (Engelhardt et al, 1990) was modified using mutagenous PCR (Dulau et al, 1989). Therefore, a forward primer was used 15 for the introduction of a new start methionine after the cleavage site of the signal peptide within a *Nco*I site (5'-AAT AGA ATT CCA TGG GGA CAC CTG CAG CTC CC-3') while the reverse primer introduced a stop codon between the putative extracellular part and the transmembrane region followed by a *Sa*II site (5' CCC AGT GTC GAC AGC CTA AAT GAT CCC 20 C-3'). The PCR product was digested with *Nco*I and *Sa*II, cloned into a pET11d expression vector (Novagen) and the proposed sequence was confirmed. The final construct was propagated in BL21(DE3) (Grodberg and Dunn, 1988). For the overexpression of Fc γ RIIb a single colony of the transformed bacteria was inoculated in 5ml LB medium containing 100 μ g 25 ampicillin per ml (LB-Amp100) and incubated overnight at 37°C. The culture was diluted 200-fold in LB-Amp100 and incubation was continued until an OD600 of 0.7-0.9 was achieved. The overproduction of the protein was induced by adding IPTG to a final concentration of 1 mM. After a growing period of 4 hours the cells were harvested by centrifugation (30 min, 4000 x g) and resuspended in sonification buffer (30 mM sodium phosphate, 300 mM sodium chloride, 0.02% sodium azide, pH 7.8). After 30 addition of 0.1 mg lysozyme per ml suspension and incubation for 30 min

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at room temperature the sonification was performed on ice (Branson Sonifier, Danbury, CT; Macrotip, 90% output, 80% interval, 15 min). The suspension was centrifuged (30 min, 30,000 x g) and resuspended with a Dounce homogenizer in sonification buffer containing 0.5% LDAO. The 5 centrifugation step and resuspension in LDAO containing buffer was repeated once before this procedure was repeated twice without LDAO. The purified inclusion bodies were stored at 4°C.

1.2 Refolding and purification of soluble human Fc γ RIIb (shFc γ RIIb)

10

The purified inclusion bodies were dissolved to a protein concentration of 10 mg/ml in 6 M guanidine chloride, 100 mM 2-mercaptoethanol and separated from the insoluble matter by centrifugation. The refolding was achieved by rapid dilution. Therefore, one ml of the inclusion body solution 15 was dropped under stirring within 15 hours into 400 ml of the refolding buffer (0.1 M TRIS/HCl, 1.4 M arginine, 150 mM sodium chloride, 5 mM GSH, 0.5 mM GSSG, 0.1 mM PMSF, 0.02% sodium azide, pH 8.5, 4°C). Afterwards, the mixture was stirred for 2-3 days until the concentration of free thiol groups was reduced to 1 mM by air oxidation as measured 20 according to Ellman (Ellman, 1959). The solution was dialyzed against PBS and sterile filtered before it was concentrated 10-fold in a stirring cell equipped with a 3kD MWCO ultrafiltration membrane. The protein solution was applied to a hIgG sepharose column (50 mg hIgG per ml sepharose 4B). Unbound protein was washed out with 50 mM TRIS pH 8.0 before elution 25 of Fc γ RIIb by pH jump (150 mM sodium chloride, 100 mM glycine, 0.02% sodium azide, pH 3.0). The eluate was immediately neutralized with 1 M TRIS pH 8.0. The Fc γ RIIb containing solution was concentrated and subjected to gel filtration on a Superdex-75 column equilibrated with crystallization buffer (2 mM MOPS 150 mM sodium chloride, 0.02% sodium 30 azide pH 7.0). The fractions containing Fc γ RIIb were pooled, concentrated to 7 mg/ml and stored at -20°C.

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1.3 Equilibrium gel filtration experiments

A Superdex75 column was connected to FPLC and equilibrated with PBS containing 10 µg shFcRIIb per ml. Human Fc fragment was solved to a
5 concentration of 1 µg/10 µl in the equilibration buffer and injected. The resulting chromatogram yielded a positive peak comprising the complex of the shFcγRIIb and the Fc fragment while the negative peak represents the lack of receptor consumed from the running buffer for complex formation.

10 1.4 Crystallization and data collection

Initial crystallization trials employing a 96 condition sparse matrix screen (Jancarik and Kim, 1991) were performed in sitting drops at 20 °C using the vapor diffusion method. Occuring crystals were improved by changing the
15 pH as well as the salt, precipitant and additive concentration. Diffraction data from suitable crystals was collected on an image plate system (MAR research) using graphite monochromated CuK_α radiation from a RU200b rotating anode generator (Rigaku) operated at 50 kV and 100 mA. The reflections were integrated with the program MOSFLM (Leslie, 1997) and
20 subsequently the data was scaled, reduced and truncated to obtain the structure-factor amplitudes using routines from the CCP4 program suite (Collaborative Computational Project, 1994).

25 1.5 Summary of expression, purification and refolding of shFcγRIIb

The extracellular part of FcγRIIb was expressed in high levels under the control of a T7 promoter in the T7 RNA polymerase positive E. coli strand BL21/DE3 (Grodberg & Dunn, 1988). The protein was deposited in inclusion bodies, which were employed in the first purification step. The isolation of
30 the inclusion bodies was started with an intense combined lysozyme/sonification procedure to open virtually all cells which would otherwise contaminate the product. The subsequent washing steps with the detergent

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LDAO, which has excellent properties in solving impurities but not the inclusion bodies itself already yielded a product with a purity of >90% (Fig. 1).

- 5 This product was used for refolding trials without further purification. The inclusion bodies were dissolved in high concentration of 2-mercaptoethanol and guanidine to ensure the shift of covalent and non-covalent aggregates to monomers. This solution was rapidly diluted with refolding buffer to minimize contacts between the unfolded protein molecules which would
10 otherwise form aggregates. The use of arginine in the refolding buffer prevents the irreversible modification of side chains as often recognized with urea. After addition of the protein to the refolding buffer, the solution was stirred at 4 °C until the concentration of free thiol groups was reduced to 1 mM, which was absolutely necessary as earlier dialysis resulted in an
15 inactive product. In a second purification step the dialyzed and refolded FcγRIIb was bound to immobilized hIgG to remove minor fractions of E. coli proteins and inactive receptor. The protein was eluted with a pH jump and immediately neutralized. After this affinity chromatography step shFcγRIIb is essentially pure except for a minor contamination resulting from the
20 coeluting IgG which leached out of the matrix even after repeated use (Fig. 1). The IgG as well as receptor multimers which are not visible in the reducing SDS-PAGE could easily be removed by gel filtration. Parallel to the removal of the contaminants in this step the buffer is quantitatively exchanged. This procedure ensures a defined composition of the protein
25 solution as even slight variations can cause irreproducibility of the crystallization attempts or even inhibit the formation of crystals. Overall 6 mg pure protein could be gained per litre E. coli culture, which is about 10 % from the FcγRIIb content of the inclusion bodies.
- 30 N-terminal protein sequencing revealed the identity with the expected sequence H₂N-GTPAAP without detectable contamination. ESI-MS analysis showed that the final material used in crystallization trials is homogenous

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with respect to size. From the primary sequence the molecular weight was calculated to 20434 Da, which corresponds to 20429 Da found by mass spectroscopy. The discrepancy lies within the error of the instrument, and no additional peak for a species containing the leading methionine is found.

5

The crystallization of shFc_yRIIb was performed in sitting drops using the vapor diffusion method. Initial trials with a sparse matrix screen (Jancarik & Kim, 1991) resulted already in small crystalline needles. Subsequent optimization of the preliminary crystallization condition by varying 10 precipitant, salt, their concentration and pH led to the isolation of three different crystal forms. Orthorhombic crystals grew from mixture of 1.5 μ l reservoir solution (33% PEG2000, 0.2 M sodium acetate, pH 5.4) with 3 μ l of the protein solution. They appeared within 3 days and reached their final size of approximately 80 μ m x 80 μ m x 500 μ m after one week. These 15 crystals diffracted to 1.7 \AA . Crystals could also be grown in two other space groups from reservoir solution containing 26% PEG8000, 0.2 M sodium acetate, pH 5.6, 5 mM Zn(OAc)₂, 100 mM sodium chloride (hexagonal form) and 26% PEG8000, 0.2 M NaOAc, pH 5.6, 10% (v/v) 1,4-Dioxan, 100 mM sodium chloride (tetragonal form). These crystals were 20 of suitable size for X-ray analysis but diffracted only to 2.7 \AA and 3.8 \AA for the tetragonal and hexagonal crystal form respectively (Table 1).

Fc_yRII was expressed in *E. coli* which, besides the comparatively low production costs and the availability, has several advantages especially 25 when the glycosylation performed by mammalian cells is not necessary for the function of the protein as in the case of Fc_yRII where IgG binding occurs independently of carbohydrate attachment (Sondermann et al, 1998A). In *E. coli* a homogenous product can reproducibly be generated, which is in contrast to the expression in mammalian cells where batch 30 dependent variances are often observed. In such a system the product is for several days exposed to proteases at temperatures of more than 30 °C. In contrary, the expression of the protein in *E. coli* under the control of the

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strong T7 promoter at 37°C frequently leads to the formation of protease inaccessible inclusion bodies. A further advantage of the expression in bacteria is that the material could be considered to be free of pathogenic germs, which might derive from employed fetal calf serum or the cell line 5 itself. In mammalian expression particular care must be taken during the purification of the target protein because potential effective hormones or growth factors might be copurified. One case where the effects of sFc γ R were ascribed to a TGF β 1 contamination is already reported (Galon et al, 1995).

10

1.6 Purification

15

The purification procedure is straightforward. It consists of three steps which can easily be performed in a single day. The protein is obtained in a pure form and in high yields and could even be obtained in considerable quality without the expensive IgG affinity column. The success of such a protocol would depend on the careful preparation of the inclusion bodies, as most of the impurities can be eliminated already in the first purification step.

20

1.7 Characterization

25

The purified Fc γ RIIb was characterized by SDS-PAGE and isoelectric focussing as well as N-terminal sequencing and mass spectroscopy. Thus, the material can be considered pure and homogeneous with respect to its chemical composition, but the intriguing question whether the receptor is correctly folded remains to be discussed. All cysteins are paired, since no free thiol groups are detected with Ellman's test. The material is monomeric and eludes with the expected retention time in peaks of symmetrical shape 30 from a size exclusion chromatography column. Furthermore, Fc γ RIIb binds to IgG sepharose, recombinant Fc γ RIIb from E. coli is active because it specifically binds IgG.

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1.8 Crystallization

The orthorhombic crystal form of Fc γ RIIb diffracted X-rays to a resolution of 1.7 Å, which is a drastic improvement compared to previously reported 5 crystals of the same molecule derived from insect cell expression (Sondermann et al, 1998A). These crystals diffracted to 2.9 Å and were of space group P3₁21. Thus, the glycosylation of the insect cell derived receptor influences the crystallization conditions. Instead of the trigonal space group, three different crystal forms are found. After a possible 10 solution of the structure these crystal forms will help identify artificial conformations of the protein due to crystal contacts.

Fc γ Rs do not exhibit any sequence similarity to other proteins but due to a conserved cystein spacing they are affiliated to the immunoglobulin super 15 family. Consequently, we tried to solve its structure by molecular replacement, but extensive trials using IgG domains from a variety of molecules failed. Thus the structure of Fc γ RIIb has to be solved by the methods of multiple isomorphous replacement.

20 We have shown for the first time that Fc γ RIIb can be obtained in an active form from E. coli. This is the basis for crystallographic investigations that will soon, due to the already gained crystals of exceptional quality, result in the structure solution of this important molecule. The structure will provide information on the IgG binding site and provide a starting point for 25 the knowledge based design of drugs that interfere with recognition of the ligand by its receptor. Furthermore, because of the high homology between Fc γ RIIb and other FcRs including Fc ϵ RIa it seems possible that these molecules can be produced in the same way, which would provide valuable material for the ongoing research.

30

Example 2

- 19 -

2.1 Methods

Protein chemistry

Recombinant soluble human Fc γ RIIb was expressed in E.coli, refolded
5 purified and crystallized as described elsewhere (Sondermann et al, 1998B). Briefly, the putative extracellular region of hFc γ RIIb2 (Engelhardt et al, 1990) was overexpressed in E. coli. Inclusion bodies were purified by lysozyme treatment of the cells and subsequent sonification. The resulting suspension was centrifuged (30 min 30,000 x g) and washed with buffer
10 containing 0.5% LDAO. A centrifugation step and resuspension in LDAO containing buffer was repeated once before this procedure was repeated twice without LDAO. The inclusion bodies were solved in 6 M guanidine hydrochloride and the protein was renatured as described. The dialyzed and filtrated protein solution was applied to a hIgG sepharose column and
15 eluted by pH jump. The concentrated neutralized fractions were subjected to size-exclusion chromatography on a Superdex-75 column (26/60, Pharmacia).

Crystallization

20 Crystallization was performed in sitting drops at 20°C using the vapor diffusion technique. Crystallization screens were performed by changing pH, salt, precipitant and additives. The final crystals used for data collection were grown in 33% PEG2000, 0.2 M sodium acetate, pH 5.4 (orthorhombic form) 26% PEG8000, 0.2 M sodium acetate, pH 5.6, 10% (v/v)
25 1,4-dioxane, 100 mM sodium chloride (tetragonal form), and 26% PEG8000, 0.2 M sodium acetate, pH 5.6, 5mM ZN(OAc)₂, 100 mM sodium chloride (hexagonal form). The insect cell derived protein was crystallized in 32% PEG6000, 0.2 M sodium acetate, pH 5.3.

30

Preparation of heavy-atom derivatives

- 20 -

The heavy-atom derivatives were prepared by soaking the crystals in the crystallization buffer containing 2 mM platinum(II)-(2,2'-6,2''terpyridinium) chloride for 24 hours or 10 mM uranylchloride for 8 days.

5 **X-ray data collection**

Diffraction data was collected on an image plate system (MAR research) using graphite monochromated CuK_α radiation from a RU200b rotating anode generator (Rigaku) operated at 50 kV and 100 mA. The reflections were integrated with the program MOSFLM 5.50 (Leslie, 1997) and 10 subsequently the data was scaled and truncated to obtain the structure-factor amplitudes using routines from the CCP4 program suite (Collaborative Computational Project, 1994).

Structure determination

15 The structure was solved with the standard procedures of the MIR method. From the large number of soaks carried out with different heavy-atom components only the two compounds yielded interpretable Patterson maps. The heavy-atom positions for each derivative were determined from difference Patterson maps and initial phases were calculated. Cross-phased 20 difference Fourier maps were used to confirm heavy atom positions and establish a common origin for the derivatives. Anomalous data were included to discriminate between the enantiomers. The heavy atom parameters were further refined with the program MLPHARE from the CCP4 package leading to the statistics compiled in Table 2. An electron-density 25 map was calculated to a resolution of 2.1 Å and the phases were improved further by solvent flattening and histogram matching with the program DM from the CCP4 suite. The resulting electron density map was of sufficient quality to build most of the amino acid residues. Model building was performed with O (Jones et al, 1991) on an Indigo2 work station (Silicon 30 Graphics Incorporation). The structure refinement was done with XPLOR (Brünger et al, 1987) by gradually increasing the resolution to 1.7 Å using the parameter set of Engh and Huber (Engh & Huber, 1991). When the

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structure was complete after several rounds of model building and individual restraint B-factors refinement ($R_{\text{fac}} = 29\% / R_{\text{free}} = 36\%$), 150 water molecules were built into the electron density when a Fo-Fc map contoured at 3.5σ coincided with well defined electron density of a 2Fo-Fc map contoured at 1σ . The resulting refinement statistic is shown in Table 3.

2.2 Structure determination

The crystal structure of recombinant soluble human Fc_yRIIb was solved by multiple isomorphous replacement (MIR) to 1.7 \AA resolution, since a structure solution by molecular replacement with isolated domains of the Fc fragment from human IgG1 (Huber et al, 1976, PDB entry 1fc1; Deisenhofer, 1981) failed. The putative extracellular part of the receptor (amino acid residues 1-187 as depicted in SEQ ID NO:2) was used for crystallization trials (Sondermann et al, 1998B) while the model contains the residues 5-176 as the termini are flexible and not traceable into the electron density. Additionally, the model contains 150 water molecules and the refinement statistics are summarized in Table 2. The structure contains a cis proline at position 11. None of the main chain torsion angles is located in disallowed regions of the Ramachandran plot. The fully refined model was used to solve the structure of the same protein in crystals of space group P4₂2₁2 and of the glycosylated form derived from insect cells in crystals of space group P3₁21 (Table 2).

The polypeptide chain of Fc_yRIIb folds into two Ig-like domains as expected from its affiliation with the immunoglobulin super family. Each domain consists of two beta sheets that are arranged in a sandwich with the conserved disulfide bridge connecting strands B and F on the opposing sheets (Fig. 3). Three anti-parallel β -strands (A1, B, E) oppose a sheet of 5 β -strands (C', C, F, G, A2), whereby strand A1 leaves the 3-stranded β -sheet and crosses over to the 4-stranded anti-parallel sheet adding the short parallel 5th strand A2. The arrangement of secondary structure

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elements as well as their connectivity is identical in both domains of the Fc γ RIIb and a rigid body fit of one domain onto the other revealed a r.m.s. distance of 1.29 Å of 67 matching C α atoms.

5 The domains are arranged nearly perpendicularly to each other enclosing an angle of 70 degrees between their long axes forming a heart-shaped overall structure. This arrangement results in an extensive contact region between the domains (Fig. 4). Residues from strand A2 and from the segment linking A2 and A1 of the N-terminal domain intermesh with residues of strands A1 and B from the C-terminal domain. This region is tightly packed and the interaction is strengthened by several hydrogen bonds resulting in a rigid arrangement. This is confirmed by the conservation of the structure in three different space groups. In orthorhombic, tetragonal and hexagonal (insect cell derived) crystal forms a deviation of less than 2° in the interdomain 10 angle is found.
15

2.3 Overall structures

The structure of recombinant human Fc γ RIIb derived from E.coli was solved 20 by MIR to 1.7 Å resolution from orthorhombic crystals. An essentially identical structure is found in tetragonal and with protein derived from insect cells in hexagonal crystals. In all three structures the last nine residues of the polypeptide chain were found disordered. The flexibility of the C-terminal linker region between the structured core of the molecule and 25 the transmembrane part may be functionally relevant to allow some reorientation of the receptor to enhance the recognition of the Fc parts in immunocomplexes.

2.4 Homologue receptors

30

The Ig domains found in the Ig super family of proteins are characterized by a beta sandwich structure with a conserved disulfide bridge connecting two

- 23 -

strands of the opposing sheets. The typical arrangement of 3 and 4 anti parallel beta strands that form a sandwich as found in Fc γ RIIb occurs also in the T cell receptor, Fc fragment, CD4 or the Fab fragment. A structural alignment of the individual Ig domains of these molecules with the two domains of Fc γ RIIb shows a common, closely related structure. The relative arrangement of the domains, however, is not related in these molecules and covers a broad sector. Despite the structural similarity between Ig domains from different molecules and the strikingly low r.m.s. deviation of C α atoms that result when the two domains of Fc γ RII are superimposed, no significant sequence similarity is found (Figs. 5a and 5b). A structure-based sequence alignment shows a conserved hydrophobicity pattern along the sequence of the domains, together with, beside the cysteins, only few identical amino acid residues. We first prepared a structure-based alignment of the two C-terminal domains of the IgG1 heavy chain and the Fc γ RIIb and added the sequences of the other related Fc γ R and the Fc ϵ RIa domains. This shows that the sequences of the three domain Fc γ RI and the two domain receptors are compatible with the hydrophobicity pattern of Ig domains and several conserved amino acid residues are revealed. Firstly, the different domains of an FcR are more related to each other than to Ig domains from other molecules of the Ig super family. Secondly, the N-terminal domains of the receptors relate to each other as the second domains do. Thirdly, the sequence of the third domain of Fc γ RI shows features from both groups of domains. Taken together, we confirm the affiliation of the FcRs to the Ig super family and speculate that all FcR-domains originate from a common ancestor, an ancient one domain receptor that acquired a second domain by gene duplication. Further divergent development of such a two domain receptor resulted in the present diversity, including Fc γ RI that acquired a third domain.

Conservation of these amino acid residues that contribute to the interdomain contact in Fc γ RIIb in the alignment are a hint to a similar domain arrangement in different receptors. In Table 4 the residues

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contributing with their side chains to the interdomain contact (Fig. 4) are compiled for Fc γ RIIb together with the corresponding amino acid residues in other receptors according to the structure-based sequence alignment of Fig. 5b. Except for Asn15, which is not conserved between the FcRs, the involved residues are identical or conservatively replaced providing strong support for a similar structure and domain arrangement in all FcRs.

2.5 The contact interface to IgG

Limited information about the interactions of FcRs with their ligands is available from mutagenesis studies (Hogarth et al, 1992; Hulett et al, 1994; Hulett et al, 1995). By systematically exchanging loops between the β -strands of Fc γ RIIa for Fc ϵ RIa amino acid residues the B/C, C'/E and F/G loops of the C-terminal domain were evaluated as important for ligand binding (Fig. 3, Fig. 5b). In the structure model these loops are adjacent and freely accessible to the potential ligand. Additionally, most of the amino acid residues in these loops were exchanged for alanines by single site mutations which resulted in a drastic alteration of the affinity of Fc γ RIIa to dimeric human IgG1. Also, the single amino acid exchange Arg 131 to His in the C-terminal domain (C'/E loop) in the high responder/low responder polymorphism, which alters the affinity of the Fc γ RIIa to murine IgG1, points to that region. Thus, the amino acid residues in this area are either important for ligand binding or the structural integrity of that region. Here, the structure shows a clustering of the hydrophobic amino acid residues Pro 114, Leu 115 and Val 116 in the neighbourhood of Tyr 157. This patch is separated from the region Leu 159, Phe 121 and Phe 129 by the positively charged amino acid residues Arg 131 and Lys 117 which protrude from the core structure (Fig. 5b).

2.6 Glycosylation

- 25 -

In the sequence of Fc γ RIIb three potential N-glycosylation sites are found. All three sites are on the surface of the molecule and are accessible. They are located in the E/F loops (N61 and N142) of both domains and on strand E (N135) of the C-terminal domain (Fig. 3, Fig. 6). Since the material used for the solution of this structure was obtained from *E. coli*, it does not contain carbohydrates, while the FcRs isolated from mammalian cells are highly glycosylated. The three potential glycosylation sites are located rather far from the putative IgG binding region, and non-glycosylated Fc γ RIIb binds human IgG, suggesting a minor role of glycosylation in binding. This was confirmed by the structure of the Fc γ RIIb produced in insect cells which is glycosylated (Sondermann et al, 1998A). Except for a 2° change of the interdomain angle possibly due to different crystal contacts, no differences between the glycosylated and unglycosylated protein structures were found. The three glycosylation sites are only optionally used as shown by SDS-PAGE where the material appears in 4 bands. No additional electron density for those sugars was found a consequence of chemical and structural heterogeneity.

2.7 The modeled complex

20

The newly solved structure of Fc γ RIIb complements the information gained from the structure of the Fc fragment and the available biochemical data regarding the Fc γ R:IgG complex.

25

While diverse biochemical information concerning the binding site of Fc γ RIIb (see above) is available, only limited data exists regarding the contact area contributed by the antibody. The IgG isotypes are closely related and exhibit graded affinities to FcRs. However, they still carry too many amino acid exchanges for the determination of the binding site and the preparation of IgG mutants is tedious. The only available information results from experiments with Fc γ R bearing cells on which bound immunocomplexes

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could be displaced with protein A (Ades et al, 1976), suggesting an at least partially overlapping binding site of protein A and Fc γ RIIb on the antibody.

With the structures of both constituents at hand we attempted to model
5 the Fc γ RII:IgG complex using the program FTDock (Gabb et al, 1997). FTDock uses Fourier correlation theory for evaluation of the shape and electrostatic complementarity of the complex component surfaces. In the hands of the authors the program has produced good results in predicting complex structures, but in some cases additional biochemical information
10 on the location of the contact area was needed to exclude false positive solutions.

Without applying additional restrictions concerning the region of the contact surface between Fc γ RIIb and the Fc fragment, the calculations resulted in
15 a single solution clearly scoring above the rather constant background. The program predicted a complex structure with the B/C, C'/E and F/G loops of the Fc γ RIIb domain 2 contributing to the contact site as predicted by the mutagenesis experiments. The only observed interaction of the N-terminal domain with the Fc fragment is via E19 that forms a salt bridge to a lysine
20 of the CH₂ domain. Some involvement of residues of the N-terminal domain in complex formation is expected since the N-terminal domain of Fc ϵ RIa cannot be exchanged against the corresponding domain of Fc ϵ RIa (Hulett et al, 1995) without losing the ligand binding capability of the receptor.

25 From the predicted interaction a model of the membrane bound complex between IgG and Fc γ RIIb is proposed (Fig. 7). Two Fc γ RIIb bind into the cleft between the third and the fourth domain of the IgG heavy chains employing the 2-fold symmetry of the Fc fragment. Protein A (Deisenhofer et al, 1978; Deisenhofer, 1981) as well as protein G (Sauer-Eriksson et al,
30 1995) and the neonatal FcR (Burmeister et al, 1994) bind to a surface region around the exposed hydrophobic residue Ile 253 of the Fc fragment. Fc γ RIIb binds to a region in the vicinity consistent with the competitive

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binding of protein A and Fc_yR to the antibody. The 2:1 stoichiometry between Fc_yRIIb and Fc fragment in the complex could be shown in equilibrium gel filtration experiments (Sondermann et al, 1998A).

5 The complex can be positioned upright on the membrane, with the truncated C-termini of Fc_yRIIb oriented towards the membrane. The N-terminal domain of the receptor lies parallel to the membrane between the Fab arms when the complex is viewed along the Fc fragment. If the FcRs have evolved from a common one domain receptor we expect that the
10 amino acid residues of the N-terminal domain that correspond to the binding region of the C-terminal domain form a second putative binding site. The corresponding surface region is accessible in the proposed complex and forms a large uncharged patch with a hydrophobic ridge comprising amino acid residues Pro 47, Leu 45, Phe 40, Leu 75, Pro 3, Pro 2 and Ala 1 (Fig.
15 6b). This region might represent a binding site for other ligands that have been discussed for Fc_yRIIb to explain the signalling capabilities of its soluble form.

Thus the modeled complex structure is consistent with the available
20 biochemical data.

Fig. 1: 15% reducing SDS PAGE showing the purification of sFc_yRIIb
Lane 1: Molecular weight marker. Lane 2: E. coli lysate before induction.
Lane 3: E. coli lysate 1 h after induction. Lane 4: E.coli lysate 4 h after
25 induction. Lane 5: Purified inclusion bodies of sFc_yRIIb. Lane 6: Eluate of
the hIgG affinity column. Lane 7: Pooled fractions of the gel filtration
column.

Fig. 2: Equilibrium gel filtration
30 1 µg hFc solved in 10 µl equilibration buffer (10 µg sFc_yRIIb/ml PBS) was applied to a size exclusion chromatography column and the absorbance of the effluent was measured (280 nm) as a function of time. The injected Fc

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fragment forms a complex with the sFc_yRIIb in the equilibration buffer (t = 22min). The negative peak of consumed sFc_yRIIb is observed at t = 26 min.

5 **Fig. 3: Overall structure of human sFc_yRIIb**

Stereo ribbon representation of the sFc_yRIIb structure. The loops supposed to be important for IgG binding are depicted in red with some of the residues within the binding site and the conserved disulfide bridge in ball and stick representation. The potential N-glycosylation sites are shown as green balls. The termini are labeled and the β -strands are numbered consecutively for the N-terminal domain in black and for the C-terminal domain in blue. The figure was created using the programs MOLSCRIPT (Kraulis, 1991) and RENDER (Merritt and Murphy, 1994).

15 **Fig. 4: Interdomain contacts**

The figure shows a close-up on the residues involved in the interdomain contacts of sFc_yRIIb. The amino acid residues of the N-terminal domain are depicted blue and the residues of the C-terminal domain yellow. The model is covered by a 2Fo-Fc electron density contoured at 1 σ obtained from the 20 final coordinates. Hydrogen bridges between the domains are represented by white lines. The figure was created using the program MAIN (Turk, 1992).

25 **Fig. 5a: Superposition of the two Fc_yRIIb domains and the CH2 domain of human IgG1**

Both domains of Fc_yRIIb and the CH2 domain of hlgG1 were superimposed. The N-terminal domain is depicted in blue, the C-terminal domain in red and the CH2 domain of hlgG1 in green. The respective termini are labeled and the conserved disulfide bridges are depicted as thin lines.

30

Fig. 5b: Structure based sequence alignment of the sFc_yRIIb domains with domains of other members of the FcR family

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- The upper part of the figure shows the structure based sequence alignment of the Fc γ RIIb and hlgG1 Fc fragment domains performed with the program GBF-3D-FIT (Lessel & Schomburg, 1994). Amino acid residues with a $C\alpha$ distance of less than 2.0 Å in the superimposed domains are masked: lilac for matching residues between the Fc fragment domains; yellow for residues in the Fc γ RIIb domains; and green when they can be superimposed in all four domains. The β -strands are indicated below this part of the alignment and are labeled consistent with Figure 3.
- 5 for matching residues between the Fc fragment domains; yellow for residues in the Fc γ RIIb domains; and green when they can be superimposed in all four domains. The β -strands are indicated below this part of the alignment and are labeled consistent with Figure 3.
- 10 The lower part of the figure shows the alignment of the amino acid sequences from the other Fc γ Rs and the homologue Fc ϵ RIa to the profile given in the upper part of the figure using routines from the GCG package (Genetics Computer Group, 1994). The upper and lower row of numbering refer to the N- and C-terminal domains of Fc γ RIIb. The conserved cysteins 15 are typed in magenta and the potential glycosylation sites in blue. Identical residues within the first domain are masked orange, those in the second domain pink and green when the residues are conserved within both domains. The less conserved third domain of Fc γ RI is aligned between the first and the second domains. Red arrows point to residues that are involved 20 in side chain contacts between the first and the second domain while blue arrows depict residues that are relevant for IgG binding. The figure was produced with the program ALSCRIPT (Barton, 1993).

Fig. 6: The putative binding sites of Fc γ RIIb

- 25 Solid surface representations of Fc γ RIIb as produced with GRASP (Nicholls et al, 1991), the color coding is according to the relative surface potential from negative (red) to positive (blue). Fig. 6a shows the molecule as in Fig. 3 by a rotation of about 90° counter-clockwise around the vertical. In Fig. 6b the molecule is rotated 90° clockwise around the same axis. Both views 30 show the putative binding regions on the C-terminal (Fig. 6a) and the N-terminal domain (Fig. 6b). The amino acid residues discussed in the text are labeled.

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Fig. 7: Model of the Fc γ R-IgG complex

The cartoon shows a complete complex of two Fc γ RIIb binding one antibody as suggested by the program FFTDOCK. The heavy chains of the antibody are depicted in red and green and the light chains in yellow. The blue atoms represent the C-terminal domain of sFc γ RIIb while the white ones represent the N-terminal domain. A blue column connects the receptor to the membrane instead of the flexible linker region that remained invisible in the electron density. The image was produced with the program POVRAY.

10

Fig. 8: Alignment of the amino acid sequence of the extracellular parts of Fc γ R and Fc ϵ RIa

Figure 8 shows an alignment of amino acid sequences of the extracellular parts of some Fc γ Rs and Fc ϵ RI.

15

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Table 1: Crystallographic results

The obtained preliminary crystallographic data are shown in this table.

	Orthorhombic	Tetragonal	Hexagonal
Space group	P2 ₁ 2 ₁ 2 ₁ , [19]	P4 ₂ 2 ₁ 2 [94]	P3 [143]
Unit cell dimensions	a = 40.8 Å, b = 50.9 Å, c = 80.5 Å, α = 90°, β = 90°, γ = 90°	a = 85.7 Å, b = 85.7 Å, c = 63.4 Å, α = 90°, β = 90°, γ = 90°	a = 80.9 Å, b = 80.9 Å, c = 157.0 Å, α = 90°, β = 90°, γ = 90°
R _{merge}	5.8%	9.8%	13.6%
Resolution	1.7 Å	2.7 Å	3.8 Å
Unique	18,040	6,616	7,210
Completeness	89.1%	97.1%	63.0%
Multiplicity	3.5	4.4	1.3
V _m , molecules per asymmetric unit, solvent content	2.09 Å ³ /Da, 1 mol., 41% solvent	2.91 Å/Da, 1 mol, 58% solvent	2.97 Å/Da, 5 mol, 59% solvent

Table 2: Data collection statistics

Derivative	Space Group	No. of unique reflections	Multiplicity	Resolution (Å)	Completeness (overall/last shell) (%/%)	R _m (%)	No. of sites	Phasing power
NATI	P2 ₁ 2 ₁ 2 ₁ .	18009	3.6	1.74	92.9/86.4	5.5		
NATI	P4 ₂ 2 ₁ 2	6615	4.5	2.70	97.1/94.3	10.1		
NATI-Baculo	P3 ₁ 2 ₁	3545	2.5	3.0	93.0/98.9	14.4		
UOAc	P2 ₁ 2 ₁ 2 ₁ .	7722	4.2	2.1	96.8/95.7	7.3	1	1.79
PtPy	P2 ₁ 2 ₁ 2 ₁ .	5520	3.9	2.3	89.7/49.6	10.5	1	1.39

$$R_m = \sum |I_h - <I_h>| / \sum |I_h>$$

Phasing power: <F_H>/E, where <F_H> = $\sum (F_H^2/n)^{1/2}$ is the r.m.s. heavy atom structure amplitude.

E = $\sum [(F_{PHC} - F_{PH})^2/n]^{1/2}$ is the residual lack of closure error with F_{PH} being the structure factor amplitude and F_{PHC} = |F_P + F_H| the calculated structure factor amplitude of the derivative.

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Table 3: Refinement statistics

5	Resolution range (Å)	8.0 - 1.74 Å
	No. of unique reflections ($F > 0\sigma(F)$)	16252
10	R factor R_{free}	19.4 27.9
	No. of atoms per asymmetric unit	
	protein	1371
	solvent	150
15	Rms deviation from ideal geometry bond length (Å) bond angle (°)	0.009 2.007
	Average B factors (Å²)	
	protein main chain	18.8
	protein side chain	25.2
	solvent	36.7
20	Rms deviation of bonded B factors (Å²)	4.1

* R_{free} : 5% of the reflections were used as a reference data set and were not included in the refinement.

Table 4: Residues that contribute to the interdomain contact via side chains

25	FcyRIIb	FcyRIIa	FcyRIII	FcyRI	FceRIa
	Asn15	Asn	Ser	Ser	Arg
	Asp20	Asp	Asp	Glu	Glu
	Gln91	Gln	Gln	Gln	Gln
30	His108	His	His	His	His
	Trp110	Trp	Trp	Trp	Trp

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SEQ ID NO:1

GTPAAPPKAV LKLEPQWINV LQEDSVTLTC RGTQSPESDS IQWFHNGNLI PTHTQPSYRF
 5 KANNNDSGEY TCQTGQTSVS DPVHLTVLSE WLVLQTPHLE FQEGETIVLR CHSWKDKPLV
 KVTFFQNGKS KKFSRSDPNF SIPQANHSHS GDYHCTGNIG YTLYSSKPVT ITVQAP...S
 SSPMGII

SEQ ID NO:2

10

AVPQKPK VSLNPPWNRI FKGENVTLTC NGNNFFEVSS TKWFHNGSLS EETNSSLNIV
 NAKFEDSGEY KCQHQQVNES EPVYLEVFSD WLLLQASAEV VMEGQPLFLR CHGWRNWVDVY
 KVIYYKDGEA LKYWYENHNI SITNATVEDS GTYYCTGKVW QLDYESEPLN ITVIKAPREK
 YWLQ (F)

15

SEQ ID NO:3

aacagaa ttccATGggg acacctgcag
 181 ctcggggaaa ggctgtgctg aaactcgagc cccagtggat caacgtgctc caggaggact
 20 241 ctgtgactct gacatgccgg gggactcaca gccctgagag cgactccatt cagtggttcc
 301 acaatggaa tctcattccc acccacacgc agcccagcta caggttcaag gccaacaaca
 361 atgacagcgg ggagtacacg tgccagactg gccagaccag cctcagcgcac cctgtgcac
 421 tgacagtgt ttctgagtgg ctggtgctcc agaccctca cctggagttc caggagggag
 481 aaaccatcg tctgaggtgc cacagctgga aggacaagcc tctggtaag gtcacattct
 25 541 tccagaatgg aaaatccaag aaatttccc gttcgatcc caacttctcc atcccacaag
 601 caaaccacag tcacagtggt gattaccatt gcacaggaaa cataggctac acgctgtact
 661 catccaagcc tgtgaccatc actgtccaag ctcccagctc ttccaccatg gggatcattT
 721 AGgctgtcga cactggg

30

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SEQ ID NO:4

gatggccata tggcagtcccct

121	cagaaaaccta aggtctcctt gaaccctcca tggaaatagaa tatttaaagg agagaatgtg
5 181	actcttacat gtaatggaa caattcttt gaagtcagtt ccaccaaatg gttccacaat
241	ggcagcctt cagaagagac aaattcaagt ttgaatattt tgaatgccaa atttgaagac
301	agtggagaat acaaatgtca gcaccaacaa gttaatgaga gtgaacctgt gtacctggaa
361	gtcttcagtg actggctgct ccttcaggcc tctgctgagg tggatgatggaa gggccagccc
421	ctcttcctca ggtgcattgg ttggaggaac tggatgtgt acaagggtat ctattataag
10 481	gatggtgaag ctctcaagta ctggtatgag aaccacaaca tctccattac aaatgccaca
541	gttgaagaca gtggAACCTA ctactgtacg ggcaaaagtgt ggcagctgga ctatgagtct
601	gagccccctca acattactgt aataaaagct ccgcgtgaga agtactggct acaatttttag
661	<u>gatccattt</u>

15

SEQ ID NO:5

human Fc γ RIIb2

1	ggctgtgact gctgtgtctt gggcgccact cgctccaggg agtgcattggaa atcctgtcat
20 61	ttttacctgt ccttgccact gagagtgact gggctgactg caagtcccc cagccttggg
121	gtcatatgtct tctgtggaca gctgtgtat tcctggctcc tggatgtggg acacctgcag
181	ctcccccaaa ggctgtgtctt aaactcgagc cccagtgat caacgtgtc caggaggact
241	ctgtgactct gacatgccgg gggactcaca gccctgagag cgactccatt cagtggttcc
301	acaatggaa ttcatttccc acccacacgc agccagacta cagttcaag gccaacaaca
25 361	atgacagcgg ggagtacacg tgccagactg gccagaccag cctcagcgac cctgtgcattc
421	tgacagtgtctt ctctgagtgg ctggctcc agacccctca cctggagttc caggagggag
481	aaaccatgt gctgaggtgc cacagcttggaa aggacaagcc tctggatcaag gtcacattct
541	tccagaatgg aaaatccaag aaattttccc gttcgatcc caacttctcc atcccacaag
601	caaaccacag tcacagtggt gattaccatt gcacaggaaa cataggctac acgtgtact
30 661	catccaagcc tggaccatc actgttcaag ctccctgtc ttcaccgtt gggatcatttgc
721	tggctgtggt cactggatt gctgttagctt ccattgttgc tgctgttagtgc gccttgcatt
781	actgcaggaa aaagcggatt tcagccatc ccactaatcc tggatgttgc gacaaagtgg
841	gggctgagaa cacaatcacc tatttacttc tcatgcaccc ggatgtctg gaagagcctg
901	atgaccagaa ccgtattttgc tctccattgtt cttgcattgg gatttgagaa gaaatcagag
35 961	aggaaagatc tggatgttcc tggcctaaat tcccattggg gaggacaggg agatgtgtca
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1141	ttgacatcaa ggctttccg ttccacatcc acacagccaa tccaaatataat caaaccactg
1201	ttatataacag ataatacgaa cttggaaat gcttatgttca caggttacgt gagaacaatc
40 1261	atgttaatct atatgatttca agaaatgttca aataatgttca acctcttacca gcacattaa
1321	agtgattgtt tctgggtgtt aaaaatttttgc atgattttttca ttttcttataa
1381	agatcatata ttacttttata aataaaacat tataaaaac

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SEQ ID NO:6

human Fc_εRIα

1 agatctcagc acagtaagca ccaggagtcc atgaagaaga tggctcctgc catggaatcc
5 61 cctactctac tgtgtgttagc cttactgttc ttgcgtccag atggcggttt agcagtcct
121 cagaaaccta aggtctcctt gaaccctcca tggaaatagaa tatttaaagg agagaatgtg
181 actcttacat gtaatggaa caatttctt gaagttagtt ccaccaaataat gttccacaat
241 ggcagcctt cagaagagac aaattcaagt ttgaatattt gtaatgccaa atttgaagac
301 agtggagaat acaaatgtca gcaccaacaa gttaatgaga gtgaacctgt gtacctggaa
10 361 gtcttcagtg actggctgct cttcaggcc tctgctgagg tggatggaa gggccagccc
421 ctcttcctca ggtccatgg ttggaggaac tggatgtgt acaagggtgat ctattataag
481 gatggtaag ctctcaagta ctggtatgag aaccacaaca tctccattac aaatgccaca
541 gttgaagaca gtggaaccta ctactgtac ggcaaaatgtt ggcaagctgga ctatgagtct
601 gagccccctca acattactgt aataaaaagct ccgcgtgaga agtactggct acaattttt
15 661 atccccattgt tgggtgtat tctgtttgct gtggacacag gattatttt ctcaactcag
721 cagcaggtca catttctctt gaagattaag agaaccagga aaggcttcag acttctgaac
781 ccacatccta agccaaaccc caaaaacaac tgatataatt aactcaagaa atatttgc当地
841 cattagttt ttccagcat cagcaattgc tactcaattt tcaaacacag cttgcaatat
901 acatagaaac gtctgtgctc aaggatttt agaaatgctt cattaaactg agtgaactg
20 961 attaagtggc atgtaatagt aagtgtcaa ttaacattgg ttgaaaat gagagaatga
1021 atagattcat ttattagcat ttgtaaaaga gatgttcaat ttagatct

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SEQ ID NO:7

human mRNA for high affinity Fc receptor (Fc γ RI)

1 gacagatttc actgctccca ccagcttggaa gacaacatgt ggttcttgac aactctgctc
5 61 ctttgggttc cagttgatgg gcaagtggac accacaaaagg cagtgatctc tttgcagcct
121 ccatgggtca gcgtgttcca agaggaaaacc gtaaccttgc actgtgaggt gctccatctg
181 cctgggagca gctctacaca gtggttctc aatggcacag ccactcagac ctgcaccccc
241 agctacagaa tcacctctgc cagtgtcaat gacagtgggt aatacagggt ccagagaggt
301 ctctcagggc gaagtgaccc catacagctg gaaatccaca gaggctggct actactgcag
10 361 gtctccagca gagtcttac ggaaggagaa cctctggcct tgaggtgtca tgcgtggaag
421 gataagctgg tgtacaatgt gctttactat cgaaatggca aagcctttaa gttttccac
481 tggaaattcta acctcaccat tctggaaaacc aacataagtc acaatggcac ctaccattgc
541 tcaggcatgg gaaagcatcg ctacacatca gcaggaatat ctgtcactgt gaaagagcta
601 tttccagtc cagtgtcaa tgcattgtg acatccccac tcctggaggg gaatctggtc
15 661 accctgagct gtgaaacaaa gttgctcttgcagaggcctg gtttgcagct ttacttctcc
721 ttctacatgg gcagcaagac cctgcgaggc aggaacacat cctctgaata ccaaataacta
781 actgctagaa gagaagactc tgggttatac tggtgcgagg ctgccacaga ggatggaaat
841 gtccttaagc gcagccctga gttggagctt caagtgccttgcctccagtt accaactcct
901 gtctggtttc atgtcctttt ctatctggca gtggaaataa tgtttttagt gaacactgtt
20 961 ctctgggtga caatacgtaa agaactgaaa agaaagaaaa agtgggattt agaaatctct
1021 ttggattctg gtcatgagaa gaaggttaact tccagccttc aagaagacag acattttagaa
1081 gaagagctga aatgtcagga aaaaaagaa gaacagctgc aggaaggggt gcaccggaag
1141 gagccccagg gggccacgtc gcagccggctc agtgggtggc catcgatctg gaccgtcccc
1201 tgcccacttg ctccccgtga gcactgcgtaa caaacatcca aaagttcaac aacaccagaa
25 1261 ctgtgtgtct catggtatgt aactcttaaa gcaaataaat gaactgactt caaaaaaaaaa
1321 a

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SEQ ID NO:8

human Fc γ RIIA

1 cccaaatgtc tcagaatgta tgtcccagaa acctgtggct gcttcaaccca ttgacagttt
 5 61 tgctgctgct ggcttctgca gacagtcaag ctgcagctcc cccaaaggct gtgctgaaac
 121 ttgagcccccc gtggatcaac gtgctccagg aggactctgt gactctgaca tgccaggggg
 181 ctcgcagccc ttagagcgac tccattcagt ggttccaccaa tgggaatctc attcccaccc
 241 acacgcagcc cagctacagg ttcaaggcca acaacaatga cagcggggag tacacgtgcc
 301 agactggcca gaccgcctc agcgaccctg tgcatactgac tggctttcc gaatggctgg
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 20 961 tggtcataact ctcagcttgc tgatggatga caaaaagagg ggaattgtta aaggaaaaatt
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 25 1261 acctacccccc aaaaaacaat tatgttaattt aaaaccaacc gattgcctt atttgcttc
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 1741 ctctgtggat ccctactgct ggtttctgcc ttctccatgc tgagaacaaa atcacctatt
 1801 cactgcttat gcagtcggaa gctccagaag aacaaagagc ccaattacca gaaccacatt
 35 1861 aagtctccat tgtttgcct tgggatttga gaagagaatt agagaggtga ggtatctggta
 1921 tttcctggac taaattccct tgggaaagac gaaggatgc tgcaggcttcca aaagagaagg
 1981 actcttccag agtcatctac ctgagttccca aagctccctg tcctgaaagc cacagacaat
 2041 atggtcccaa atgactgact gcaccttctg tgcctcagcc gttcttgaca tcaagaatct
 2101 tctgttccac atccacacag ccaatacaat tagtcaaacc actgttatta acagatgttag
 40 2161 caacatgaga aacgctttag ttacaggtt catgagagca atcatgttaag tctatatgac
 2221 ttcagaaatg taaaataga ctaacctcta acaacaaatt aaaagtgatt gttcaaggt
 2281 gatgcaatta ttgatgaccc ttttatttt tctataatga tcatatatta cctttgtaat
 2341 aaaacattat aaccaaaac

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SEQ ID NO:9

human Fc γ RIII

1 tctttggta cttgtccact ccagtgtggc atcatgtggc agctgctctt cccaaactgct
5 61 ctgctacttc tagttcagc tggcatgcgg actgaagatc tcccaaaggc tgggtgttc
121 ctggagcctc aatggtacag cgtgctttag aaggacatgt tgactctgaa gtgccaggaa
181 gcctactccc ctgaggacaa ttccacacag tggtttcaca atgagagcct catctcaagc
241 caggcctcgta gctacttcat tgacgctgcc acagtcaacg acagtggaga gtacagggtgc
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10 361 ttgctccagg cccctcggtg ggtgttcaag gaggaagacc ctattcacct gaggtgtcac
421 agctggaaga acactgctct gcataaggc acatatttac agaatggcaa agacagggaaag
481 tattttcatc ataattctga ctccacatt cccaaagcca cactcaaaga tagcggctcc
541 tacttctgca gggggcttgt tgggagtaaa aatgtgtctt cagagactgt gaacatcacc
601 atcactcaag gtttggcagt gtcaaccatc tcatttcattctt ctccacctgg gtaccaagtc
15 661 tctttctgct tgggtatggt actccctttt gcagtggaca caggactata tttctctgtg
721 aagacaaaca tttgaagctc aacaagagac tggaggacc ataaacttaa atggagaaag
781 gaccctcaag acaaattgacc cccatccccat ggagtaata agagcagtgg cagcagcatc
841 tctgaacatt tctctggatt tgcaacccca tcatttcctcag gcctctc

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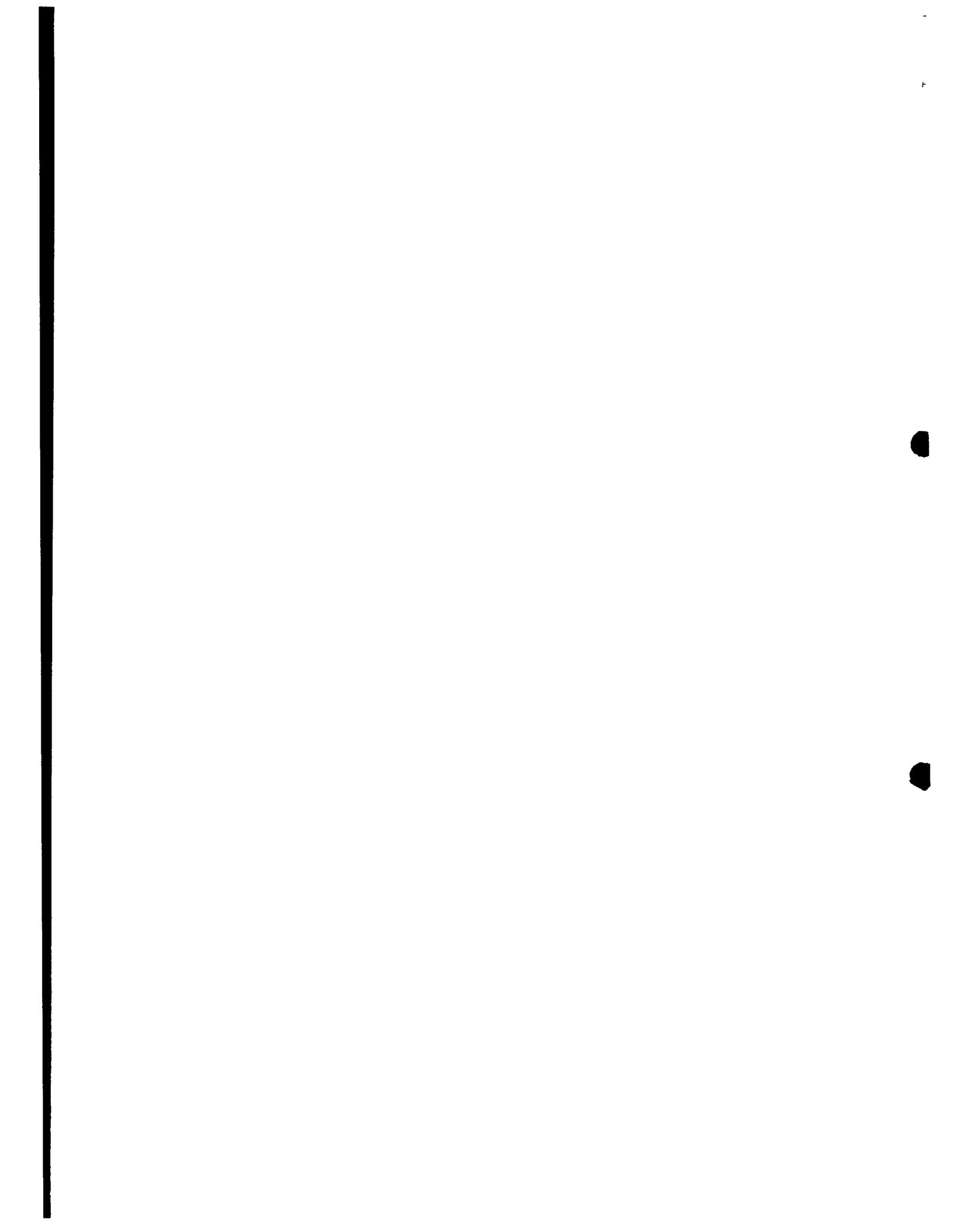
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Claims

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1. Recombinant soluble Fc receptor characterized by the absence of transmembrane domains, signal peptide and glycosylation.
- 5
2. Recombinant Fc receptor according to claim 1, wherein the receptor is a Fc γ R or a Fc ϵ R.
- 10
3. Recombinant Fc receptor according to claim 1 or 2, wherein the receptor is a Fc γ RIIb.
4. Recombinant Fc receptor according to any one of claims 1 to 3, wherein the receptor is of human origin.
- 15
5. Recombinant Fc receptor according to any one of claims 1 to 4, wherein it contains the amino acids as shown in SEQ ID NO:1 or SEQ ID NO:2.
- 20
6. Recombinant nucleic acid containing a sequence encoding a recombinant Fc receptor according to any one of claims 1 to 5.
7. Recombinant nucleic acid according to claim 6, wherein it contains a sequence as shown in SEQ ID NO:3 or SEQ ID NO:4.
- 25
8. Recombinant nucleic acid according to claim 6 or 7, wherein it additionally contains expression control sequences operably linked to the sequence encoding the recombinant Fc receptor.
- 30
9. Recombinant nucleic acid according to any one of claims 6 to 8,

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wherein it is contained on a prokaryotic expression vector, preferably a pET vector.

10. Host cell characterized by the presence of a recombinant nucleic acid according to any one of claims 6 to 8.
- 5 11. Host cell according to claim 10,
 wherein it is a prokaryotic host cell, preferably an E. coli cell.
- 10 12. Process for the determination of the amount of antibodies of a certain type in the blood, plasma or serum of a patient, characterized by the use of a recombinant soluble Fc receptor according to any one of claims 1 to 5 in an immunoassay and determination of the presence of FcR-antibody complexes.
- 15 13. Process according to claim 12, wherein the immunoassay is an ELISA and preferably a sandwich assay.
- 20 14. Process according to claim 12 or 13, wherein the antibodies to be determined are IgE antibodies and the recombinant soluble receptor is a Fc ϵ R.
- 25 15. Process according to claim 14 for the determination of a predisposition or manifestation of an allergy.
16. Process according to claim 12 or 13, wherein the antibodies to be determined are IgG antibodies and the recombinant soluble receptor is a Fc γ R.
- 30 17. Process for the determination of the immune status of patients with chronic diseases of the immune system, wherein a Fc receptor according to any one of claims 1 to 5 is used in a competitive

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immunoassay and the amount of the corresponding sFcRs in the blood, plasma or serum of a patient is determined.

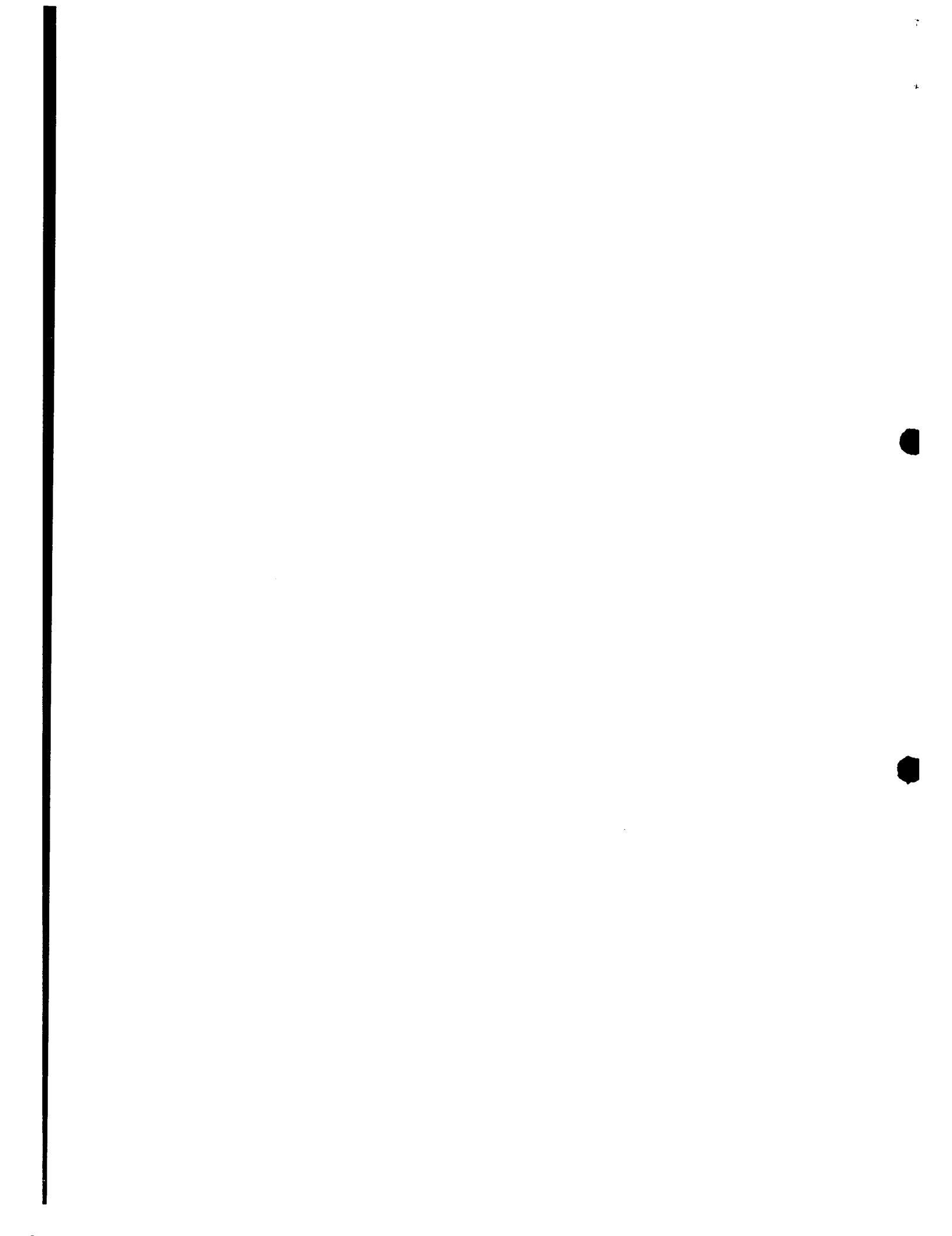
18. Process according to claim 17, wherein the chronic disease is AIDS,
5 SLE, MM or rheumatoid arthritis.
19. Use of a recombinant soluble Fc receptor according to any one of claims 1 to 5 for the screening of substances in view of their ability to act as inhibitors of the recognition and binding of antibodies to the
10 respective cellular receptors.
20. Use according to claim 19, wherein recombinant soluble Fc_yRs are used and recognition and binding of IgG antibodies is of interest.
- 15 21. Pharmaceutical composition containing as active agent a recombinant soluble FcR according to any one of claims 1 to 5.
22. Pharmaceutical composition according to claim 21 for use in the treatment or prevention of autoimmune diseases, allergies or tumor
20 diseases.
23. Pharmaceutical composition according to claim 21 or 22 for use in the treatment of AIDS, rheumatoid arthritis or multiple myeloma, containing a recombinant soluble Fc_yR preferably having the amino
25 acid sequence as shown in SEQ ID NO:1.
24. Use of a crystalline preparation of a recombinant soluble Fc receptor according to any one of claims 1 to 5 for the generation of crystal structure data of Fc receptors.
30
25. Use of crystal structure data obtained by the use according to claim 24 for the identification and preparation of Fc receptor inhibitors.

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26. Use of crystal structure data obtained by the use according to claim 24 for the identification and preparation of new antibody receptors.
27. Use according to any one of claims 24 to 26 in a computer-aided modelling program.
5
28. FcR inhibitor characterized in that it has a three-dimensional structure which is complementary to the recombinant soluble FcR according to any one of claims 1 to 5.
10
29. Pharmaceutical composition containing as active agent a FcR inhibitor according to claim 28.
15
30. Pharmaceutical composition according to claim 29 for use in the treatment or prevention of diseases which are due to overreactions or faulty reactions of the immune system.
20
31. Pharmaceutical composition according to claim 29 or 30 for the treatment or prevention of allergies, autoimmune diseases or an anaphylactic shock.
25
32. Fc receptor according to claims 1-5, bound to a solid phase.
30
33. Fc receptor according to claim 32, wherein the solid phase is a chromatography carrier material.
34. Use of a chromatography carrier material according to claim 33 for the adsorption of immunoglobulins from the blood, plasma or serum of a patient or from culture supernatants of immunoglobulin producing cells.

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35. Use according to claim 34 for the enrichment of antibodies from a patient's blood, serum or plasma or from culture supernatants of immunoglobulin producing cells for the conduction of further tests.



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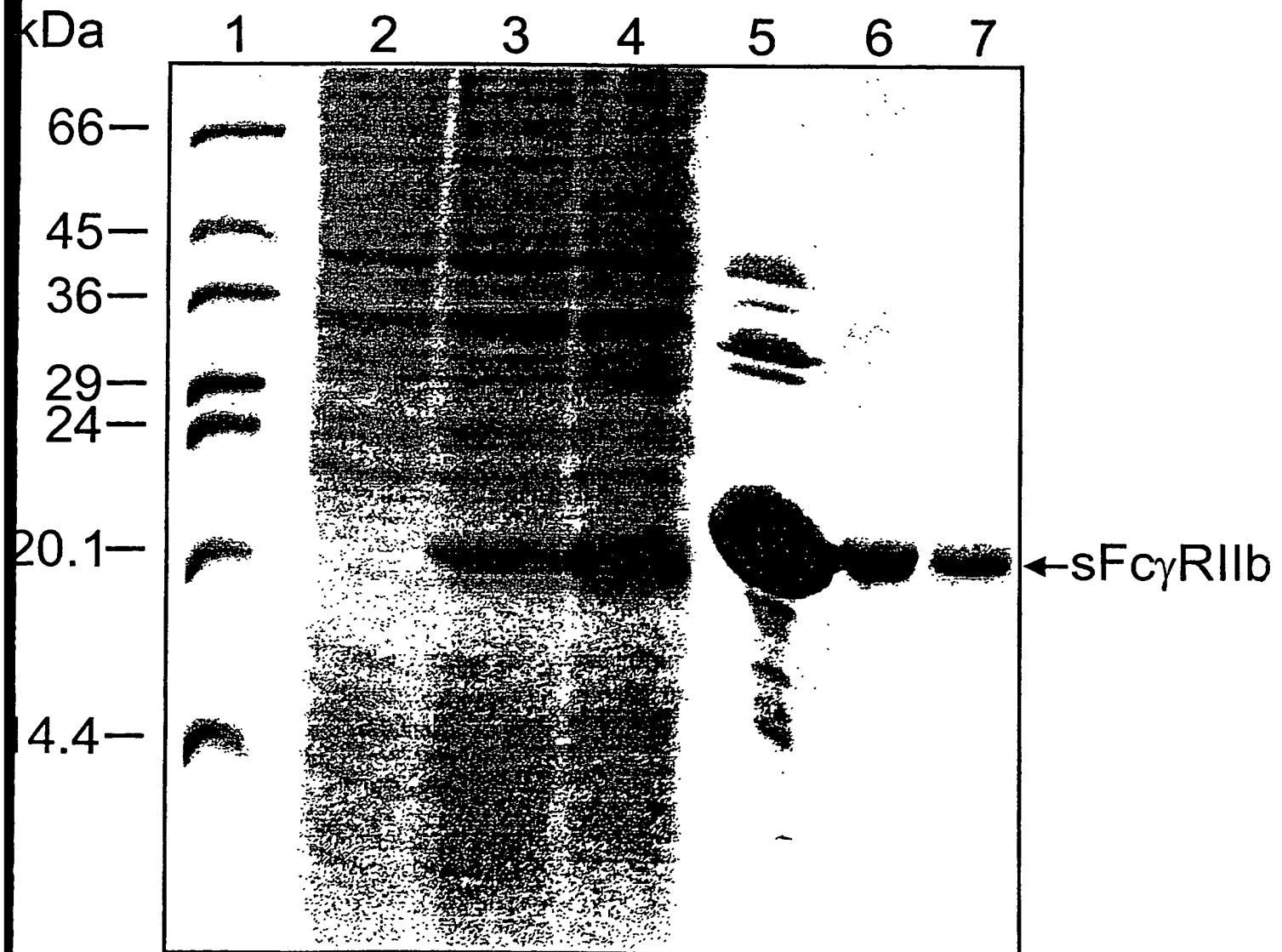


FIG. 1

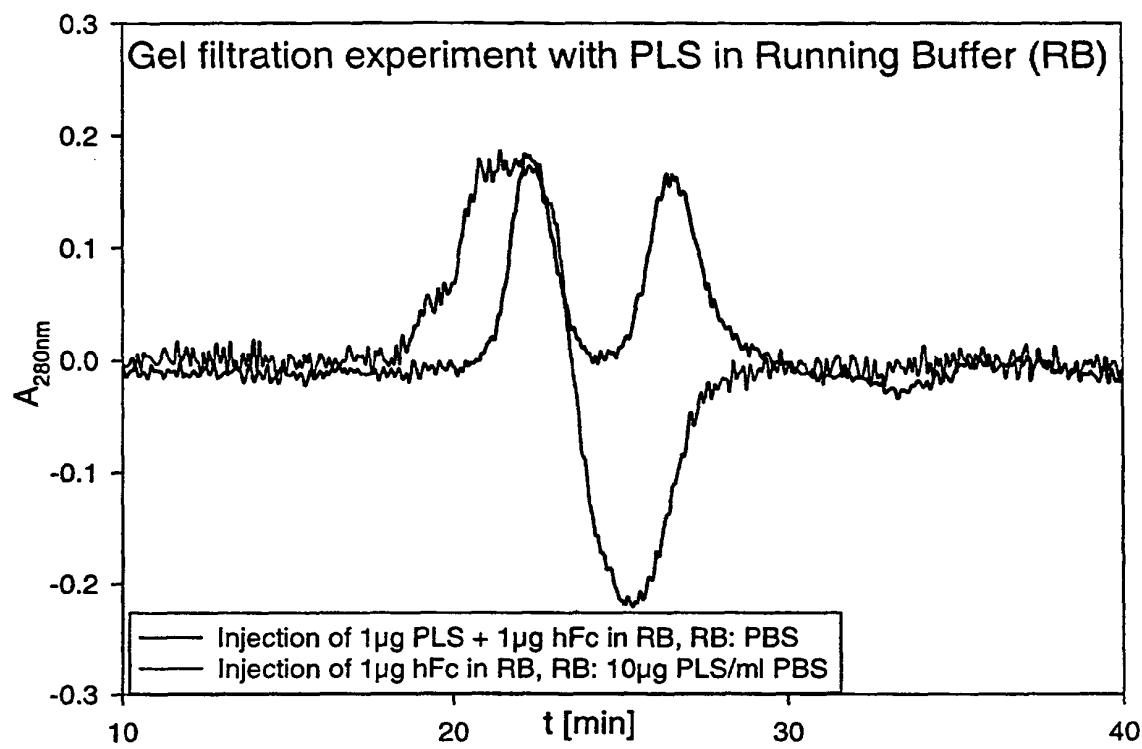


FIG. 2

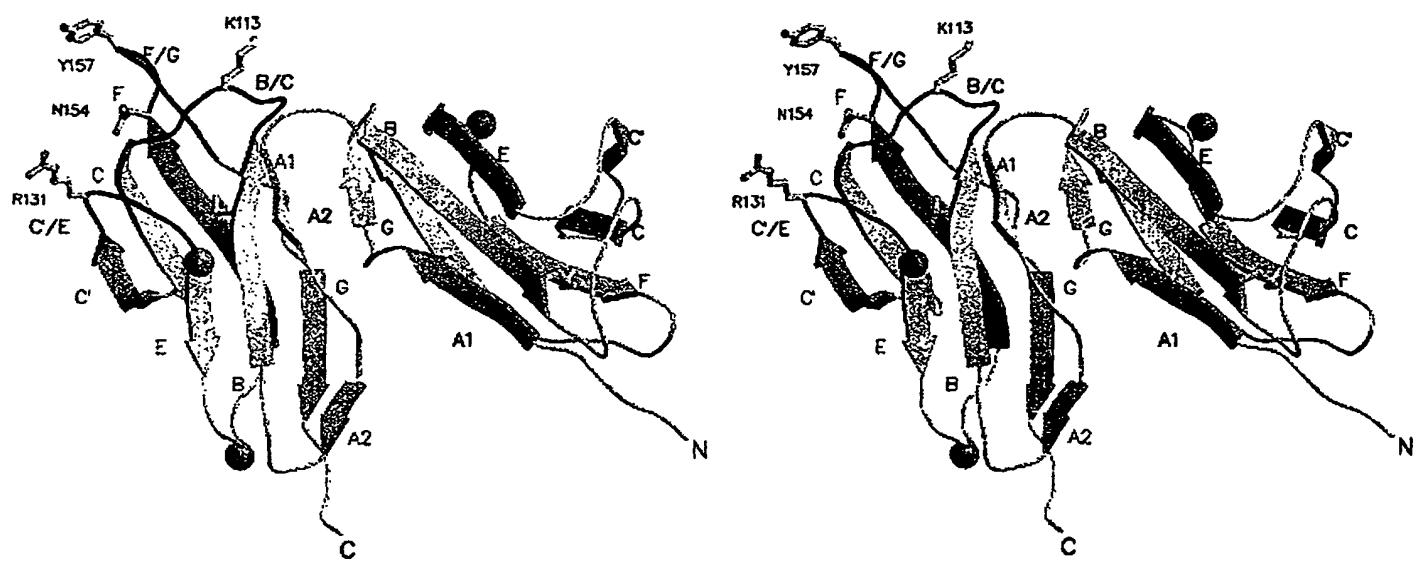


FIG. 3

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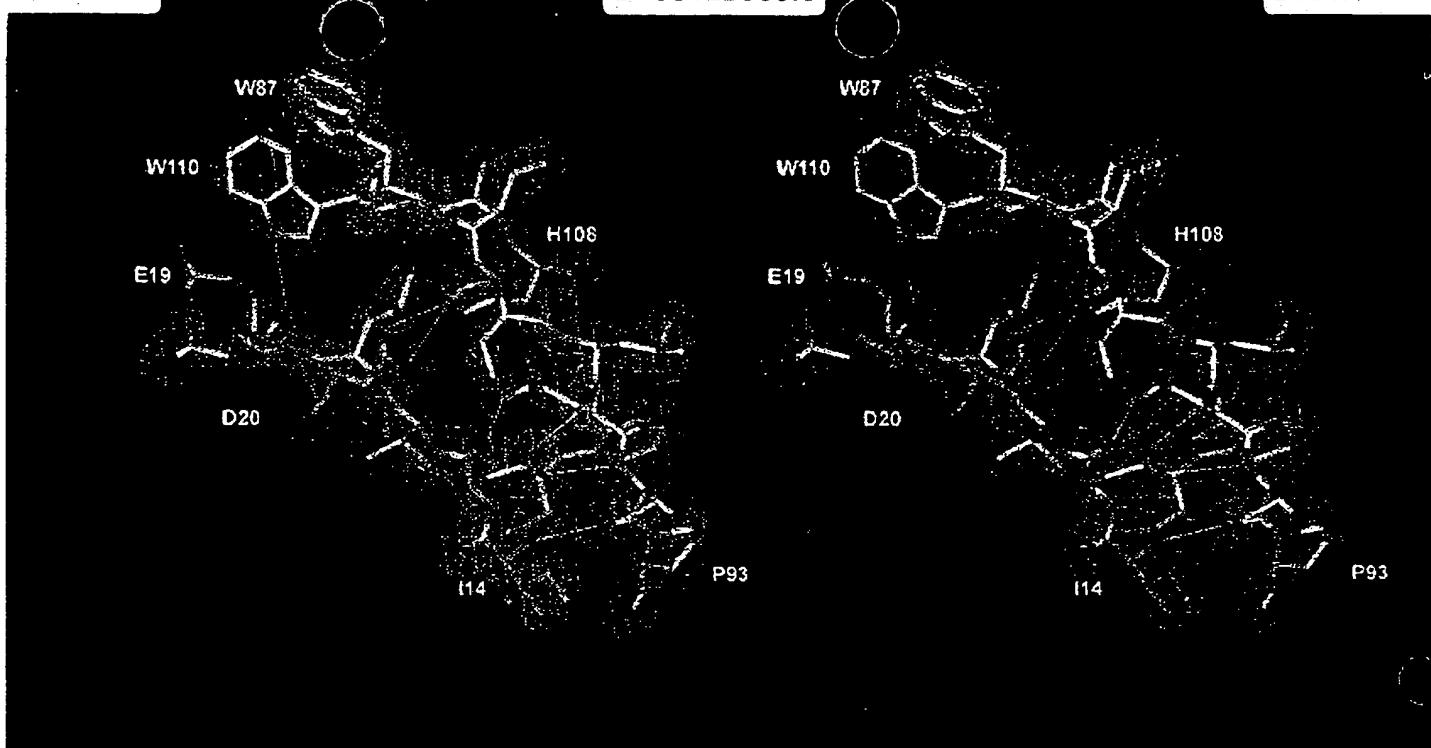


FIG. 4

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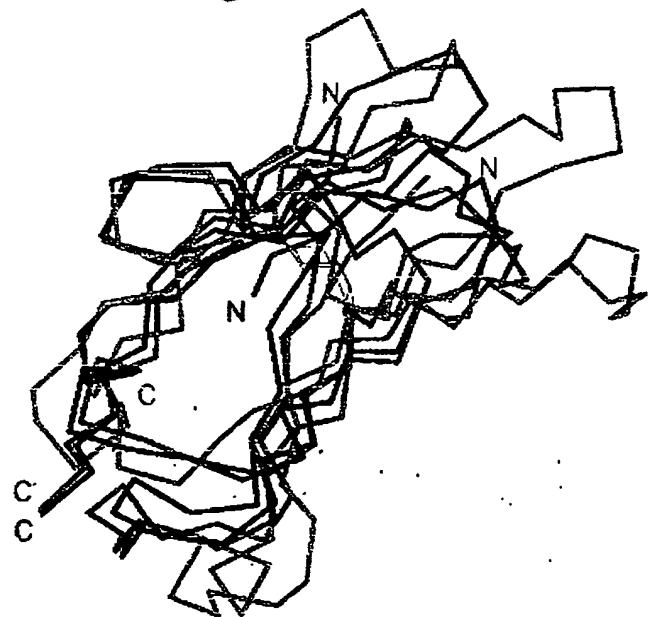
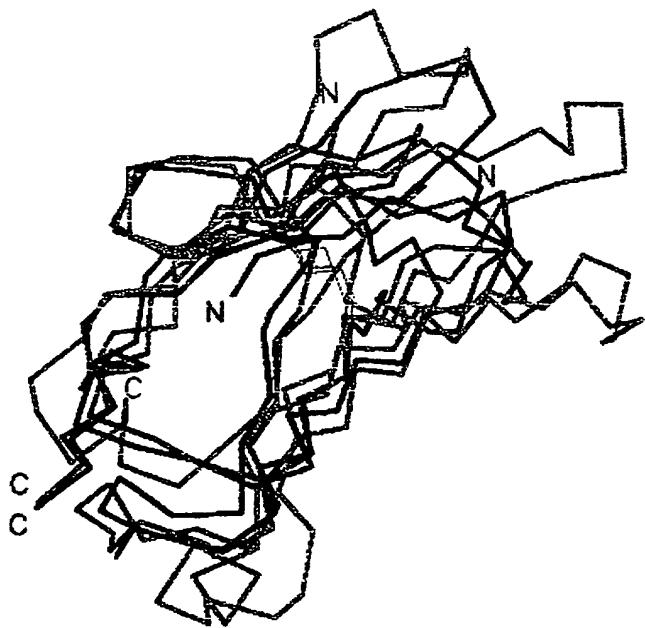


FIG. 5A

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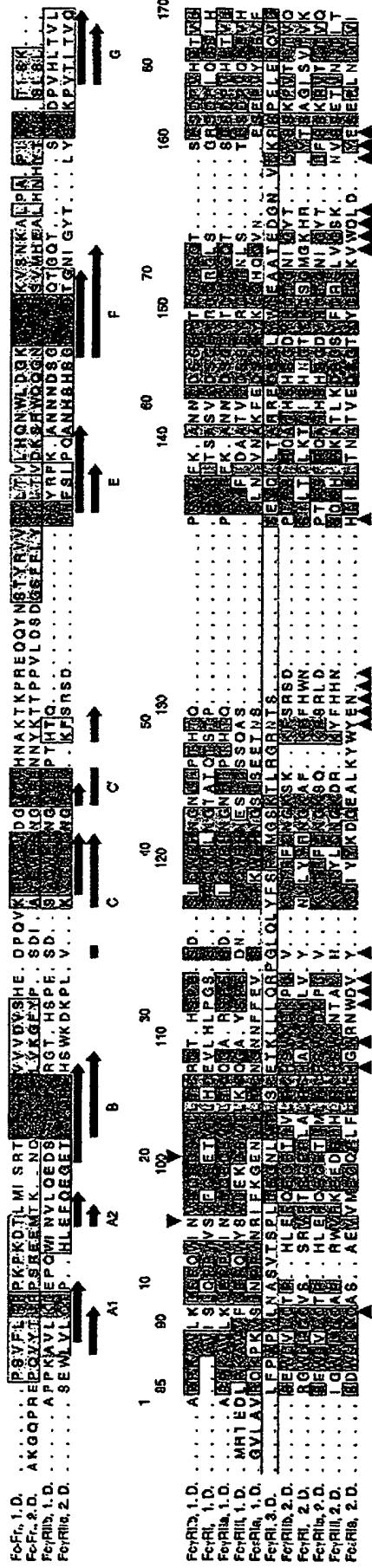


FIG. 5B

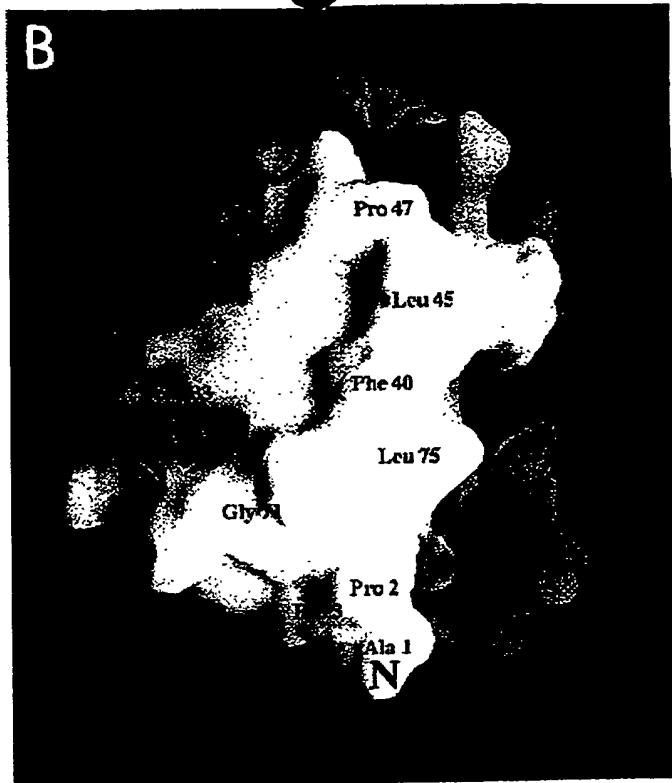


FIG. 6

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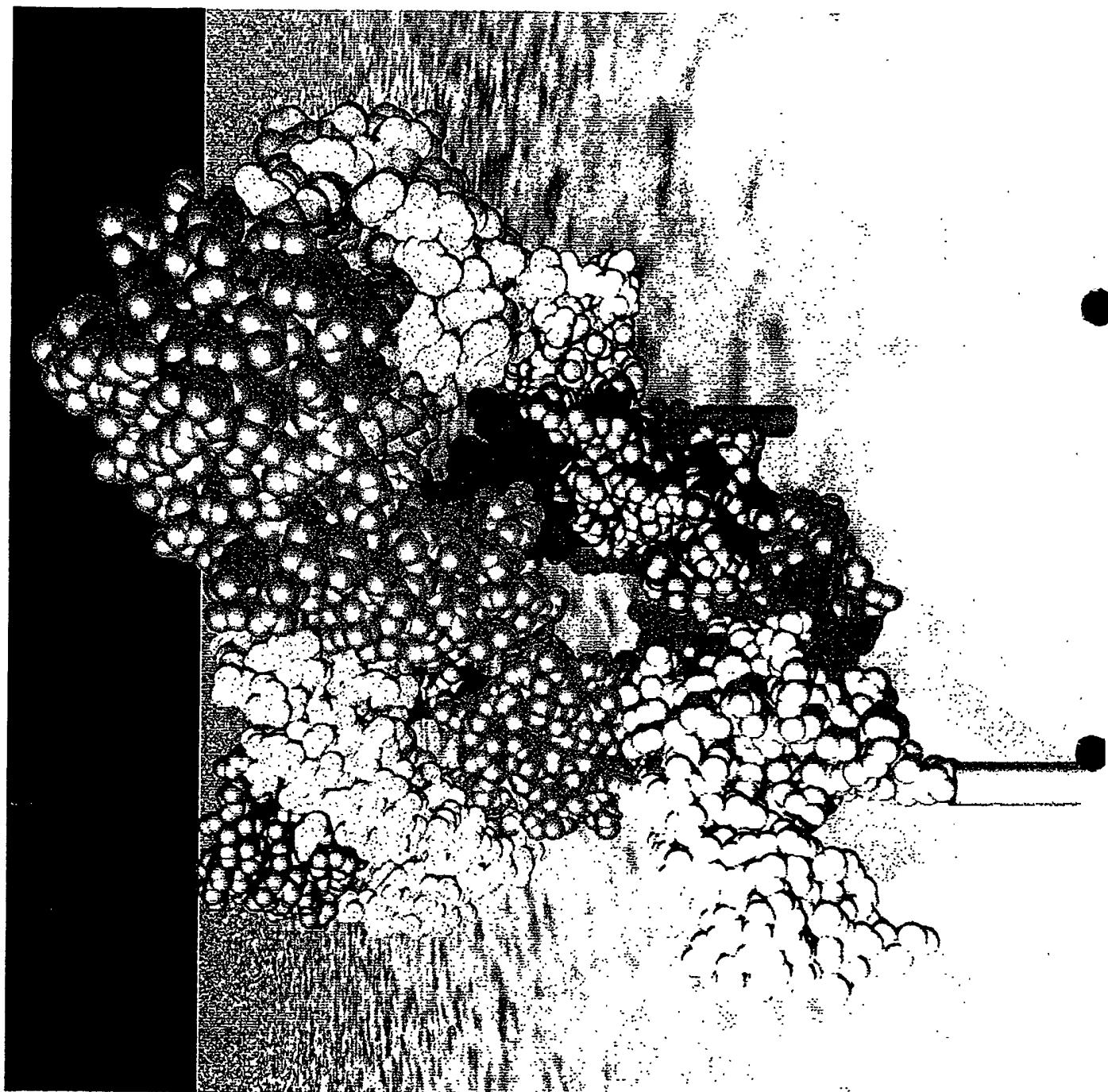


FIG. 7

Alignment of the amino acid sequence of the extracellular parts of Fc γ R and Fc ϵ RIa without signal sequence and transmembrane region

FcγRIIa FcγRIIb FcγRIII FcγRI FcϵRIa	1 SQAAAPPKAV Ikleppwinv LQEDSVTLTC QGARSPE SDS Iqwfhngnlj PtHtopsyrf KANNNDSEY TcqTgotsls Dpvhltlse wlvLQTPHLE GTPAAPPKAV Ikleppwinv LQEDSVTLTC RGTOSPE SDS Iqwfhngnlj PtHtopsyrf KANNNDSEY TcqTgotsvs Dpvhltlse wlvLQTPHLE MRTEDLPKAV VFLEPQWYSV LEKDSVTLKC QGAYSPEDS TQWFNESLI SSQASSYFID AATVNDSEY RCQTNLSTLS DPVQLEYHIG WLLQAPRWVAV ISLQOPPWYV FQEETVTLHC EVLHLPGS S QWFLNGTAT QTSTPSYRIT SASVNDSEY RCQRLGSGRS DPIQLEIHG WLLQVSSRV GVLAVPQPK VSLNPPWNRI FKGENVTILTC NGNNNFFEVSS TKWFHNGSIS ETETSSLNIV NAKFEDSEY KCQHQQVNES EPVYLEVFS D WLLQASAEV	100 50
FcγRIIa FcγRIIb FcγRIII FcγRI FcϵRIa	101 FQEGETIMLR CHSWKDPLV KVTFQNGKS QKFSLRIDPTF SIPQANSHS GDYHCTGNIG YTLFSSKPVT ITVQVPSMGS SSPMGII..... FQEGETIVLR CHSWKDPLV KVTFQNGKS KKFSRSDPNF SIPQANSHS GDYHCTGNIG YTYSSKPVT ITVQAP...S SSPMGII..... FKEEDPIHLR CHSWKNTALH KVTYLQNGKD RKYFHHSNSDF HIPKATLKD GSYFCRGLVG SKVNSSETVN ITITOGLAVS TISSESPP..... FTEGEPLMLR CHAWKDPLV NVLYYRNGKA FKFFFHWNSNL TIKTNISHN GTHCSC MG KHYRTAGIS VTVKELFPAP VLNASVTSPL LEGNLVTLSC VMEGQPLFLR CHGWRNWDDVY KVIYYKDGEA IKYIYENHNI SITNATEVD S GTYYCTGKWW QLDYESEPLN ITVIKAPREK YWLQ.....	150 200
FcγRIIa FcγRIIb FcγRIII FcγRI FcϵRIa	201 ETKILLQRPG LQLYFSFYMG SKTLGRNTS SEYQILTARR EDSGLYWCEA ATEDGNVLR SPELELQVLG LQLPTPV	250 277



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Abstract

Recombinant soluble Fc receptors according to the present invention are characterized by the absence of transmembrane domains, signal peptides and glycosylation. Such Fc receptors can easily be obtained by expressing respective nucleic acids in prokaryotic host cells and renaturation of the obtained inclusion bodies, which procedure leads to a very homogenous and pure product.

10

The products can be used for diagnostic as well as pharmaceutical applications and also for the generation of crystal structure data. Such crystal structure data can be used for the modelling of artificial molecules.

15

A further embodiment comprises coupling the Fc receptors according to the invention to solid materials like chromatography materials that can be used to separate and/or enrich antibodies.

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